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PART III] *SECTION A* [VOL. XXVI

STUDIES ON THE RECLAMATION OF INDIAN ALKALI SOILS

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AGRICULTURISTS have long recognized the important role of soil and fertilizer phosphorus in crop production. Bone, the first important source of phosphorus fertilizer, was used in Great Britain as early as 1774. Its use increased steadily and about 100 years ago England was importing such large quantities of bone from the continent of Europe that Liebig, the noted German chemist, wrote with some alarm.

" England is robbing all other countries of the condition of their fertility. Already in her eagerness for bones she has turned up the battlefields of Leipsig, of Waterloo, and of the Crimea; already from the catacombs of Sicily she has carried away the skeletons of many successive generations."

Experience has shown that many soils are deficient in phosphorus and that the use of phosphatic fertilizers materially increased crop production. Collings[1] has emphasised the role of phosphates in the maintenance of soil fertility in the following words:

" Low crop yields are more often due to a lack of phosphorus than to a lack of any other nutrient. Phosphorus has often been called the ' master key ' to agriculture. Phosphorus appears to be concerned in the production of nucleoproteins, cell division, and the rate of metabolism and

it appears that phosphorus influences the production of seed or grain more particularly than does nitrogen or potassium."

Bear[2] has stated, "Within limits, phosphate fertilizer together with potash salt and lime can be substituted for nitrogen fertilizers. Their use stimulates the nitrogen fixing bacteria, both symbiotic and non-symbiotic, to greater activity. The rank growth of clover that follows the addition of phosphate and potash resembles the application of nitrogenous fertilizers." Russell[3] has rightly stated "Phosphate supply may become a factor that will determine the course of history".

The importance of phosphates is due to the reason that phosphorus is an essential constituent of plant and animal life; the quantity in the average soil is relatively small, particularly in alkali soil, and there is no atmospheric supply of this element on which plants can grow. The importance of phosphate chemistry in alkaline calcareous soils involve largely the nature and reactions of slightly soluble calcium phosphates naturally present in the soil and of the calcium phosphates resulting from the reactions between the soil and applied phosphatic fertilizers.

The phosphatic manures are practically all compounds of phosphoric acid with lime, and as is well known four distinct combinations exist and are found in commerce: monocalcium phosphate $CaH_4(PO_4)_2$ or $Ca(H_2PO_4)_2$; dicalcium phosphate $CaHPO_4$ or $Ca_2H_2(PO_4)_2$; tricalcium phosphate $Ca_3(PO_4)_2$ and tetracalcium phosphate $Ca_4P_2O_9$. Only one, the monocalcium phosphate is soluble in water; the others give rise to extremely dilute suspension of phosphoric acid, too dilute to nourish a plant properly and hence are termed insoluble. It is well known that when water-soluble monocalcium phosphate is added to the soil, a major part is rapidly converted into insoluble form, *i.e.*, fixed by the soils and only a very small portion of the added phosphate is taken up by the plants. This phenomenon is due to the conversion of monocalcium phosphate to dicalcium, tricalcium, iron, aluminium or titanium phosphates according to the following equations:

$$CaH_4(PO_4)_2 + 2\,CaCO_3 = Ca_3(PO_4)_2 + 2H_2O + 2CO_2$$

$$CaH_4(PO_4)_2 + Ca_3(PO_4)_2 = 4\,CaHPO_4$$

$$CaH_4(PO_4)_2 + 2\,CaCO_3 = 2\,CaHPO_4 + Ca(HCO_3)_2$$

$$CaH_4(PO_4)_2 + Al_2(SO_4)_3 = 2\,AlPO_4 + CaSO_4 + 2\,H_2SO_4$$

$$CaH_4(PO_4)_2 + Fe_2(SO_4)_3 = 2\,FePO_4 + CaSO_4 + 2\,H_2SO_4$$

$$2\,CaH_4(PO_4)_2 + 3\,TiCl_4 = Ti_3(PO_4)_4 + 2\,CaCl_2 + 8\,HCl.$$

Soils vary greatly in their capacity to fix phosphates. It is well known that pH, organic matter, amount of soluble calcium and magnesium, amount and the chemical nature of the clay colloid and the amount of iron and aluminium present in the soils are all important factors affecting the phosphate fixing power of soils.

McGeorge[4] indicates that in alkaline calcareous soils the phosphate solubility increases above pH 8·5, is at a minimum within the range 7·6–8·5, and increases again below pH 7·6. Burd[5] and Gardner and Kelley[6] observed that solubility curves for phosphates in calcareous soils had a minimum near pH 7–8 with increasing solubility at higher or lower pH. Gardner and Kelley[6] showed that 1% K_2CO_3 solution increased the solubility of PO_4 in calcareous soils and indicated that their increase was due largely to the solution of calcium phosphate rather than iron or aluminium phosphate. They point out that the CO_3'' reduces the Ca^{++} to very low values. The solubility of phosphate in the slightly acid range is closely correlated with the solubility in the highly alkaline range for soils of similar composition. This indicates that the dissolving action of alightly acid and also alkaline solution is upon similar forms of calcium phosphate.

When soluble phosphates are applied to soils containing fair amounts of calcium, reversion takes place quickly to dicalcium and slowly to tricalcium phosphates. According to McGeorge,[4] the depressing effect of $CaCO_3$ on the absorption of PO_4 is due to a decreased solubility of soil phosphate and to the tendency of $CaCO_3$ to maintain a high pH. Work by McGeorge,[4] Hibbard,[7] Ensminger and Larsen,[8] Smith[9] and Hockensmith, Gardner and Goodwin[10] have shown that, within the range of 1–4% lime, PO_4 absorption by plants is roughly inversely related to the lime content of the soil. Harrison and Das[11] found that monocalcium phosphate reacts very repidly with $CaCO_3$ to form dicalcium phosphate and that this in turn reacts more slowly to form less soluble tricalcium phosphate. Austin[12] gives the reactions for the titration of monocalcium phosphate with excess of $Ca(OH)_2$ as follows:

$$3\,Ca(OH)_2 + CaH_4(PO_4)_2 = Ca_3(PO_4)_2 + Ca(OH)_2 + 4\,H_2O$$

With excess $CaCO_3$:

$$3\,CaCO_3 + CaH_4(PO_4)_2 = 2\,CaHPO_4 + Ca(HCO_3)_2 + CaCO_3.$$

This he feels, indicates that large amounts of CO_2 in calcareous soils should enhance phosphatic availability.

Some of the possible reactions of soluble phosphates with $CaCO_3$ were indicated by McGeorge and Breazeale,[13] McGeorge[4] and Boischot, Coppenet and Herbert.[14] These reactions include conversion to a less soluble precipitate of a calcium phosphate, with the $CaCO_3$ furnishing the calcium, and followed by excess Ca^{++} and CO_3'' being absorbed on the surface of calcium phosphate crystals. Russell[15] suggests that two or three years may be required in soils before dicalcium phosphate is fully converted into less soluble form. Dicalcium phosphate is not ordinarily applied alone to soils but rather in close association with soluble salts, which may well decrease the rate and amount of conversion to less soluble forms.

In general it is believed that the presence of organic matter in soils has a beneficial effect on the availability of phosphorus. Hester and Sheldon[16] obtained increased yield of beans by raising the organic matter content by the addition of peat moss in presence of superphosphate. They believe that the presence of organic matter delayed the absorption of phosphorus by iron and aluminium and thus kept it more available to the plants. Rahn[17] found that phosphatic manures are available when used in conjunction with organic matter.

Scarseth[18] reports that the humate part of organic matter may actually replace the fixed phosphate ion and thus make it more available. Gaarder,[19] Meyer[20] and Arie[21] have observed similar effects exerted by humus.

Jensen[22] found that the addition of organic matter to a soil increases the solubility of both lime and phosphoric acid from 30–100%. Baure,[23] Ramaswami Sivan[24] and Hopkins[25] observed increased availability of PO_4 on mixing rock phosphate with farm or green manures.

Puri[26] has studied the role of CO_2 in the reclamation of alkaline soils. Soils of varying degrees of alkalinity at different pH levels were prepared by neutralizing the H-soil with varying quantities of $Ca(OH)_2$–NaOH mixtures of appropriate strength. The results as expressed in terms of the percentage of sodium in total bases (Ca + Na) present in the soil are given in Table I.

The following conclusions regarding the action of CO_2 on alkali soils were drawn by Puri as a result of this study.

(*a*) There is a substantial decrease in the pH value at all degrees of alkalinity, as indicated by the percentage of NaOH in the mixtures of hydroxides.

(*b*) At low pH values, flocculation occurs at all degrees of alkalization. When the pH value is high, flocculation fails to take place at degrees of alkalinity higher than 60–80%.

TABLE I

Effect of CO_2 on soils of different degrees of alkalinity

NaOH (m.e. per 100 gm.)	Ca $(OH)_2$ (m.e. per 100 gm.)	Degree of alkalinity (%)	Initial pH	pH after passage of 1% CO_2	pH after passage of 100% CO_2	Flocculation*	pH after addition of $CaCO_3$	pH after passage of CO_2
0	12·0	0	5·80	6·02	5·20	F	..	..
1·6	6·4	20	5·86	5·67	5·26	F	6·58	5·77
3·2	4·8	40	5·86	5·86	5·40	F	6·47	5·86
4·8	3·2	60	5·95	5·80	5·40	F	6·40	5·85
6·4	1·6	80	6·00	5·86	5·25	F	7·00	6·00
8·0	0	100	6·50	5·50	5·25	F	6·90	5·90
0	18·0	0	6·00	6·38	5·02	..	..	..
3·2	12·8	20	6·20	5·55	5·40	F	6·32	5·85
6·4	9·6	40	6·40	6·20	5·40	PF	6·38	6·00
9·6	6·4	60	6·40	5·70	5·50	PF	6·43	5·87
12·8	3·2	80	6·60	5·36	4·58	PF	6·28	5·80
16·0	0	100	7·33	5·06	4·62	PF	6·38	5·90
0	50	0	8·30	7·55	5·80	F	..	..
8·0	32·0	20	8·60	7·50	5·60	F	6·90	5·80
16·0	24·0	40	8·80	6·17	5·60	F	6·60	5·80
24·0	16·0	60	9·10	7·35	5·78	PF	6·80	6·10
32·0	8·0	80	9·35	6·75	6·10	PF	7·54	5·90
40·0	0	100	9·50	6·50	6·00	X	7·20	6·18

* F = Flocculated; PF = Partly flocculated; X = No flocculation.

(*c*) In the presence of $CaCO_3$, a large amount of Na saloid is converted into Ca saloid with the production of $NaHCO_3$, which can be leached out, resulting in a substantial reduction in the degree of alkalization.

Puri has concluded that alkali soils with higher pH values cannot be reclaimed by CO_2 or by organic matter alone. Application of calcium salt along with organic matter is essential for the reclamation of such soils.

Recently Dhar[27] has developed a new concept for the reclamation of alkali soils by a mixture of organic matter and rock phosphates. According to him carbonic acid produced in the oxidation of organic matter can readily convert tricalcium phosphate to dicalcium phosphate, which can slowly supply calcium ions to the soil solution in small amounts and thus, calcium carbonate is formed by the action of alkali present in alkali soil and the calcium ions obtained from $CaHPO_4$. He is of the opinion that a mixture of organic matter and rock phosphate is more profitable in reclaiming alkali soils than a mixture of organic matter and calcium carbonate because $CaHPO_4$ is formed more readily in the former case than $Ca(HCO_3)_2$ in the latter case. Moreover, in the nitrification of proteins present in legumes, nitrous and nitric acids are formed which are profitable in the reclamation of alkali lands. They also claim that during this process of reclamation of alkali soil, the nitrogen status also improves.

Carbonic acid plays an important role in soils. It is well known that carbonic acid is a much weaker acid than phosphoric acid as is evident from their dissociation constants:

Carbonic acid: H_2CO_3: dissociation constant $= 3 \times 10^{-7}$

$$\frac{H^+ \times HCO_3'}{H_2CO_3} = 3 \times 10^{-7} \text{ (first dissociation constant)}$$

$$\frac{H^+ \times CO_3''}{HCO_3'} = 6 \times 10^{-11} \text{ (second dissociation constant)}$$

Phosphoric acid: H_3PO_4: dissociation constant $= 9 \times 10^{-3}$

$$\frac{H^+ \times H_2PO_4'}{H_3PO_4} = 1{\cdot}1 \times 10^{-2} \text{ (first dissociation constant)}$$

$$\frac{H^+ \times HPO_4''}{H_2PO_4'} = 2 \times 10^{-7} \text{ (second dissociation constant)}$$

$$\frac{H^+ \times PO_4'''}{HPO_4''} = 3{\cdot}6 \times 10^{-13} \text{ (third dissociation constant)}$$

As the dissociation constant of carbonic acid is much smaller than that of phosphoric acid, calcium carbonate is much more alkaline in its properties than calcium phosphate, although the solubility in water of both these calcium salts is practically the same as recorded in Table II.

TABLE II

Substance	Formula	Solubility in 100 parts of water
Calcium carbonate	.. $CaCO_3$	0·0013 at 0° C.
Monocalcium phosphate	.. $Ca(H_2PO_4)_2.H_2O$	4·0 at 15° C.
Dicalcium phosphate	.. $CaHPO_4.2\ H_2O$	0·028 at 0° C.
Tricalcium phosphate	.. $Ca_3(PO_4)_2$	0·0013 at 0° C.

The carbonic acid produced during the decomposition of organic matter attacks more readily the tricalcium phosphate, if present and converts it into dicalcium phosphate. The readily soluble $Ca(HCO_3)_2$ is formed with more difficulty from $CaCO_3$ under the same conditions. Although dicalcium phosphate is sparingly soluble as is evident from Table II, but in contact with water, it is slowly converted into $Ca(H_2PO_4)_2$ and $Ca_3(PO_4)_2$.

$$4\ CaHPO_4 \rightleftharpoons Ca_3(PO_4)_2 + Ca(H_2PO_4)_2.$$

In other words, dicalcium phosphate formed in the soil by the action of decomposing organic matter on tricalcium phosphate, when in contact with water partially behaves as a dilute solution of monocalcium phosphate and can reclaim alkali soils slowly. Rindell[28] has clearly shown that monocalcium phosphate is always present in water in contact with dicalcium phosphate. Thus the monocalcium phosphate formed during the hydrolysis of dicalcium phosphate can readily convert the sodium carbonate and sodium bicarbonate present in the alkali soil into sodium phosphate, calcium carbonate and calcium bicarbonate respectively as given below.

$$Na_2CO_3 + Ca(H_2PO_4)_2 = 2\ NaH_2PO_4 + CaCO_3$$

$$2\ NaHCO_3 + Ca(H_2PO_4)_2 = 2\ NaH_2PO_4 + Ca(HCO_3)_2.$$

Consequently, the harmful effect of alkali carbonates is minimised due to the formation of sodium phosphate. Simultaneously $Ca(HCO_3)_2$ produced supplies soluble calcium ions which replace sodium ions from the

exchange complex of the alkali soil, thereby leading to the reclamation of alkali soils.

As emphasised previously, humus acts as a marked adsorbent of ions positive and negative, *i.e.*, they can affect the acidity or alkalinity of a soil markedly and can act as buffers. It is clear, therefore that under ordinary condition soils rich in humus cannot be either too acid or too alkaline. Hence, there seems to be a fundamental difference between the effect of loss of humus in cold and in hot countries. If the humus is lost in cold countries, there is always a possibility of the formation of acid soils. Even in hot countries, it has been reported that laterites which are poor in humus are acidic due to the washing away of calcium and its replacement by hydrogen-ions. On the other hand in hot countries, in most cases the oxidation of humus is more quick and is greatly facilitated by the development of alkali. So once the soil in a warm country becomes alkaline and if organic matter is not added, the alkalinity may go on increasing, chiefly due to loss of humus. The loss of humus seems to be an autocatalytic reaction and the oxidation is facilitated by alkalinity. Thus the net effect is the almost complete removal of humus and most alkali soil is reduced to ashes rich in sodium carbonate. The remedy is clearly the addition of calcium ion in the system so that the soil can pass into Ca-soil, certainly increasing the amount of organic matter by addition of cowdung, green manure, leaves specially legumes.

Large amount of work on the reclamation of alkali soils have been conducted all over the world. Kelley and Thomas[29] realised the importance or organic matter in the reclamation of alkali soils and showed that chemical amendments such as gypsum or sulphur in combination with manure improve the alkali soil permanently. Mitra and Shanker[30] have shown that alkali soils from Soraon, Allahabad, which has been used in these experiments can be reclaimed permanently by the application of chemical amendments along with organic matter.

Gypsum, sulphur, sulphuric acid, ferrous and aluminium sulphates are well recognised alkali ameliorating agents, but the cost of these chemicals is beyond the reach of proverbially poor Indian cultivators. The method reported in this paper, *i.e.*, addition of a mixture of phosphatic amendment along with organic matter is definitely cheaper and superior to the classical methods of alkali soil reclamation. It not only reclaims the alkali soils, but improves the fertility status by increasing the nitrogen, humus, available phosphate and exchangeable calcium. This method reclaims

the alkali soils permanently and is suitable for the arid and semi-arid conditions.

EXPERIMENTAL

200 gm. of well powdered Soraon alkali soil, after being passed through 80 mesh sieve were taken in enamelled dishes. 13 gm. and 26 gm. sun-hemp (*Crotolaria juncea*), 9·2 and 20·6 gm. spent wash, 5 and 10 gm. of wheat straw were added in different dishes, so that the initial carbon of the mixtures were 2·47%, 4·59%, 1·23%, 2·468%, 1·24% and 2·47% respectively. Various phosphatic amendments (in a finely divided form), *i.e.*, raw bone-meal, Trichinopoly rock phosphate, North African rock phosphates A, B, C, D_1 and D_2, basic slag (Tata) and Bihar rock phosphate were applied in different doses. Calcium carbonate was used as an amendment in sun-hemp treated samples to compare its effect with basic slag (Tata), which is rich in $CaCO_3$. All the experiments were done in controlled conditions and therefore no replication was given to the treatments studied.

The soils along with organic matter and amendment were mixed very thoroughly in order to obtain homogeneous mixtures. About 50% distilled water was added to these mixtures and this moisture level was maintained throughout the experiments. The dishes were exposed to diffused light of the room and the contents were stirred from time to time to facilitate aeration and proper decomposition of the organic matter. Composite samples were taken at regular intervals and were analysed for carbon by Robinson, McLeans and William s method,[31] total nitrogen by salicylic acid reduction method,[32] exchangeable calcium by Hissink's method,[33] available phosphate by Williams' method[34] and pH.

The object of these experiments was to investigate the effect of the mixture of organic matters *plus* cheap phosphatic amendments on the reclamation of alkali soils and to find out a suitable dozc of these materials for the economic reclamation of alkali soils on a field scale.

TABLE III

Per cent. chemical composition of alkali soil from Soraon

Moisture	1·39
Loss on ignition ..	2·50
HCl insoluble	84·51
Sesquioxide	7·05
Fe_2O_3	3·38
CaO	1·06
MgO	1·31
P_2O_5	0·142
K_2O	1·10
Available P_2O_5	0·032
Total carbon	0·135
Total nitrogen	0·035
pH	10·0

TABLE IV

Per cent. chemical composition of organic matters

	CaO	MgO	K_2O	P_2O_5	Total carbon	Total nitrogen
Sun-hemp ..	2·10	0·16	1·32	0·518	37·706	1·231
Wheat straw ..	0·28	0·06	1·06	0·053	38·231	0·734
Spent wash ..	1·42	0·12	0·72	0·053	25·120	0·362

TABLE V

Per cent. chemical composition of phosphatic amendments

	Silica	Fe_2O_3	P_2O_5	K_2O	CaO	MgO	Available P_2O_5
North African rock phosphate:							
A ..	1·68	Nil	19·64	9·02	10·65	1·16	7·57
B ..	4·32	Nil	26·34	8·84	13·28	1·92	5·62
C ..	5·00	Nil	24·15	7·56	7·46	2·08	6·64
D_1 ..	2·61	Nil	19·24	1·14	6·23	1·63	3·01
D_2 ..	2·52	Nil	25·26	1·85	6·23	..	3·09
Basic slag (Tata) ..	23·66	38·90*	7·59	9·07	34·36	5·28	4·46
Trichinopoly rock phosphate	6·30	4·20	27·50	4·50	18·76	1·92	3·16
Bihar rock phosphate ..	5·52	3·98	19·56	3·25	11·62	0·71	3·00
Raw bone-meal ..	4·50	..	20·17	..	25·50	0·57	7·00

* Sesquioxides.

TABLE VI

200 gm. Soil + 13 gm. Sun-hemp + 1·0% Calcium Carbonate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·090	..	3·5	0·029	10·0
60	1·643	0·061	33·5	5·6	0·047	9·7
120	1·421	0·056	40·5	6·2	0·048	9·4
210	1·210	0·052	51·0	6·5	0·051	8·7
360	1·042	0·052	57·8	6·6	0·058	8·5

TABLE VII

200 gm. Soil + 13 gm. Sun-hemp + 2·0% Calcium Carbonate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·090	..	3·5	0·029	10·0
60	1·579	0·069	36·0	5·7	0·050	9·7
120	1·300	0·064	45·3	7·5	0·056	9·5
210	1·052	0·062	57·4	7·8	0·069	8·8
360	0·988	0·060	59·9	7·8	0·070	8·6

TABLE VIII

200 gm. Soil + 13 gm. Sun-hemp + 1·0% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·090	..	3·5	0·065	10·0
60	1·730	0·065	30·0	5·6	0·090	9·6
120	1·520	0·061	38·4	6·4	0·091	9·3
210	1·320	0·058	46·5	6·9	0·094	8·7
360	1·140	0·058	53·8	7·0	0·097	8·5

TABLE IX

200 gm. Soil + 13 gm. Sun-hemp + 2·0% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·090	..	3·5	0·096	10·0
60	1·676	0·077	32·1	5·8	0·128	9·6
120	1·404	0·072	43·3	7·6	0·135	9·2
210	1·164	0·069	53·4	8·0	0·138	8·7
360	1·042	0·067	57·8	8·2	0·140	8·5

TABLE X

200 gm. Soil + 13 gm. Sun-hemp + 1·0% Bihar Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·090	..	3·5	0·032	10·0
60	1·850	0·078	25·1	5·8	0·050	9·5
120	1·648	0·074	33·3	6·8	0·056	9·2
210	1·467	0·072	40·6	7·4	0·060	8·5
360	1·181	0·070	52·2	7·8	0·064	8·3

TABLE XI

200 gm. Soil + 13 gm. Sun-hemp + 2·0% Bihar Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·090	..	3·5	0·032	10·0
60	1·760	0·081	28·7	6·2	0·050	9·4
120	1·558	0·079	37·0	8·3	0·065	9·0
210	1·351	0·077	45·0	8·7	0·068	8·2
360	1·131	0·076	54·2	8·7	0·071	8·0

TABLE XII

200 gm. Soil + 26 gm. Sun-hemp + 1·0% Calcium Carbonate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	4·590	0·160	..	3·5	0·029	10·0
60	2·671	0·125	41·8	5·7	0·058	9·6
120	2·350	0·110	48·8	6·2	0·060	9·4
210	2·040	0·088	55·5	6·7	0·062	8·8
360	1·821	0·085	60·3	7·1	0·064	8·6

TABLE XIII

200 gm. Soil + 26 gm. Sun-hemp + 2·0% Calcium Carbonate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	4·590	0·160	..	3·5	0·029	10·0
60	2·471	0·132	46·2	5·8	0·059	9·6
120	2·011	0·120	53·2	7·5	0·071	9·4
210	1·611	0·103	64·0	8·3	0·079	8·7
360	1·605	0·095	65·0	8·6	0·084	8·6

TABLE XIV

200 gm. Soil + 26 gm. Sun-hemp + 1·0% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	4·590	0·160	..	3·5	0·065	10·0
60	2·854	0·130	37·8	5·7	0·101	9·5
120	2·641	0·118	42·5	6·3	0·101	9·3
210	2·420	0·095	47·3	6·9	0·104	8·6
360	1·975	0·084	56·9	7·5	0·108	8·3

TABLE XV

200 gm. Soil + 26 gm. Sun-hemp + 2·0% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	4·590	0·160	..	3·5	0·096	10·0
60	2·617	0·137	43·0	5·9	0·129	9·5
120	2·210	0·125	53·0	7·8	0·137	9·2
210	1·810	0·106	60·5	8·5	0·141	8·6
360	1·704	0·097	62·8	8·6	0·152	8·4

TABLE XVI

200 gm. Soil + 26 gm. Sun-hemp + 1·0% Bihar Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	4·590	0·160	..	3·5	0·031	10·0
60	3·160	0·137	31·6	5·9	0·065	9·5
120	2·870	0·126	37·4	7·0	0·070	9·1
210	2·593	0·115	43·5	7·8	0·078	8·4
360	2·154	0·101	53·1	8·5	0·083	8·2

TABLE XVII

200 gm. Soil + 26 gm. Sun-hemp + 2·0% Bihar Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	4·590	0·160	..	3·5	0·032	10·0
60	3·064	0·145	33·3	6·5	0·068	9·4
120	2·771	0·136	39·0	8·6	0·082	8·7
210	2·470	0·122	46·0	9·3	0·087	7·9
360	2·154	0·112	53·0	9·5	0·092	7·8

TABLE XVIII

200 gm. Soil + 9·2 gm. Spent Wash + 0·5% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·233	0·050	..	3·5	0·070	10·0
90	0·806	0·055	34·6	5·8	0·088	9·4
180	0·657	0·059	46·7	7·0	0·010	8·8
270	0·545	0·062	55·8	7·9	0·115	8·2
360	0·447	0·062	63·7	8·3	0·121	7·6

TABLE XIX

200 gm. Soil + 9·2 gm. Spent Wash + 1·0% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·233	0·050	..	3·5	0·105	10·0
90	0·806	0·056	34·6	5·8	0·125	9·4
180	0·614	0·061	50·0	7·0	0·134	8·7
270	0·535	0·063	55·8	7·9	0·142	8·2
360	0·438	0·063	64·5	8·4	0·148	7·5

TABLE XX

200 gm. Soil + 9·2 gm. Spent Wash + 0·5% Trichinopoly Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·233	0·050	..	3·5	0·043	10·0
90	0·824	0·054	33·8	5·7	0·075	9·6
180	0·664	0·058	46·1	6·9	0·083	8·8
270	0·560	0·060	54·6	7·5	0·091	8·3
360	0·485	0·060	60·7	7·9	0·096	7·8

TABLE XXI

200 gm. Soil + 9·2 gm. Spent Wash + 1·0% Trichinopoly Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·233	0·050	..	3·5	0·053	10·0
90	0·814	0·055	33·9	5·7	0·088	9·5
180	0·655	0·060	46·8	6·9	0·096	8·8
270	0·551	0·062	55·3	7·5	0·103	8·3
360	0·453	0·063	63·2	8·0	0·110	7·8

TABLE XXII

200 gm. Soil + 9·2 gm. Spent Wash + 0·5% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·232	0·050	..	3·5	0·056	10·0
90	0·794	0·054	35·6	5·4	0·066	9·7
180	0·644	0·056	47·8	6·7	0·075	9·3
270	0·524	0·057	57·5	7·4	0·082	8·8
360	0·435	0·057	64·7	7·8	0·090	8·4

TABLE XXIII

200 gm. Soil + 9·2 gm. Spent Wash + 1·0% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·233	0·050	..	3·5	0·077	10·0
90	0·781	0·055	36·6	5·5	0·087	9·6
180	0·631	0·057	48·7	6·7	0·109	9·1
270	0·513	0·058	58·4	7·7	0·118	8·6
360	0·438	0·058	64·5	8·2	0·128	8·2

TABLE XXIV

200 gm. Soil + 20·6 gm. Spent Wash + 0·5% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·468	0·066	..	3·5	0·066	10·0
90	1·610	0·068	34·8	5·8	0·095	9·4
180	1·356	0·069	45·6	7·0	0·114	8·7
270	1·117	0·068	50·5	7·9	0·121	8·1
360	0·914	0·068	63·0	8·4	0·127	7·5

TABLE XXV

200 gm. Soil + 20·6 gm. Spent Wash + 1·0% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·468	0·066	..	3·5	0·100	10·0
90	1·585	0·068	35·7	5·9	0·125	9·4
180	1·336	0·069	45·8	7·1	0·137	8·6
270	1·100	0·069	55·4	7·9	0·148	8·1
360	0·924	0·068	62·9	8·5	0·156	7·5

TABLE XXVI

200 gm. Soil + 20·6 gm. Spent Wash + 0·5% Trichinopoly Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·468	0·066	..	3·5	0·041	10·0
90	1·646	0·067	33·3	5·7	0·082	9·5
180	1·384	0·068	43·9	7·0	0·092	8·8
270	1·137	0·068	53·9	7·9	0·100	8·3
360	0·900	0·068	63·5	8·4	0·108	7·7

TABLE XXVII

200 gm. Soil + 20·6 gm. Spent Wash + 1·0% Trichinopoly Rock Phosphate

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·468	0·066	..	3·5	0·051	10·0
90	1·632	0·068	34·3	5·7	0·088	9·5
180	1·372	0·069	44·5	7·1	0·099	8·8
270	1·125	0·069	54·5	7·9	0·108	8·2
360	0·905	0·068	63·3	8·4	0·115	7·7

TABLE XXVIII

200 gm. Soil + 20·6 gm. Spent Wash + 0·5% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·468	0·066	..	3·5	0·053	10·0
90	1·586	0·067	35·7	5·5	0·074	9·6
180	1·332	0·067	46·0	6·9	0·084	9·2
270	1·087	0·066	56·4	7·7	0·092	8·7
360	0·915	0·066	62·9	8·1	0·100	8·2

TABLE XXIX

200 gm. Soil + 20·6 gm. Spent Wash + 1·0% Basic Slag (Tata)

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·468	0·066	..	3·5	0·073	10·0
90	1·586	0·068	35·7	5·7	0·105	9·6
180	1·320	0·068	46·5	6·9	0·117	9·1
270	1·077	0·067	56·4	7·9	0·128	8·5
360	0·895	0·067	63·8	8·4	0·135	8·0

TABLE XXX

200 gm. Soil + 5 gm. Wheat Straw + 0·5% Raw Bone-Meal

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·066	10.0
90	0·884	0·056	31·9	5·8	0·090	9·6
180	0·761	0·058	38·6	7·1	0·099	8·8
270	0·643	0·060	48·1	7·8	0·104	8·2
360	0·512	0·062	58·7	8·3	0·107	7·7

TABLE XXXI

200 gm. Soil + 5 gm. Wheat Straw + 1·0% Raw Bone-Meal

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·101	10·0
90	0·825	0·056	33·4	6·3	0·134	9·4
180	0·747	0·060	40·0	7·5	0·145	8·8
270	0·621	0·062	49·8	8·2	0·148	8·1
360	0·503	0·063	59·5	8·7,	0·150	7·6

TABLE XXXII

200 gm. Soil + 5 gm. Wheat Straw + 0·5% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·069	10·0
90	0·848	0·056	31·5	5·7	0·089	9·6
180	0·772	0·059	37·7	7·0	0·096	8·9
270	0·652	0·061	47·4	7·7	0·101	8·3
360	0·533	0·062	57·0	8·2	0·104	7·8

TABLE XXXIII

200 gm. Soil + 5 gm. Wheat Straw + 1·0% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·017	10·0
90	0·829	0·056	33·1	6·2	0·134	9·5
180	0·755	0·059	39·0	7·4	0·143	8·9
270	0·626	0·062	49·4	8·1	0·148	8·2
360	0·521	0·064	58·0	8·5	0·151	7·8

TABLE XXXIV

200 gm. Soil + 5 gm. Wheat Straw + 0·5% North African Rock Phosphate B

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·059	10·0
90	0·849	0·055	31·5	5·6	0·074	9·7
180	0·778	0·058	37·2	6·8	0·080	9·0
270	0·658	0·058	46·9	7·5	0·084	8·5
360	0·535	0·057	57·0	8·1	0·086	7·9

TABLE XXXV

200 gm. Soil + 5 gm. Wheat Straw + 1·0% North African Rock Phosphate B

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·088	10·0
90	0·832	0·055	32·8	6·1	0·107	9·6
180	0·761	0·057	38·6	7·3	0·115	9·0
270	0·630	0·059	49·2	7·8	0·119	8·4
360	0·537	0·058	56·6	8·4	0·121	7·9

TABLE XXXVI

200 gm. Soil + 5 gm. Wheat Straw + 0·5% North African Rock Phosphate C

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·065	10·0
90	0·849	0·056	31·5	5·6	0·082	9·7
180	0·778	0·058	37·2	7·0	0·088	8·9
270	0·657	0·058	47·7	7·4	0·093	8·4
360	0·535	0·058	57·0	8·1	0·095	7·8

TABLE XXXVII

200 gm. Soil + 5 gm. Wheat Straw + 1·0% North African Rock Phosphate C

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·098	10·0
90	0·830	0·056	33·0	6·3	0·120	9·5
180	0·759	0·058	38·7	7·4	0·129	8·9
270	0·627	0·060	49·4	7·9	0·134	8·3
360	0·528	0·059	57·3	8·3	0·128	7·8

TABLE XXXVIII

200 gm. Soil + 5 gm. Wheat Straw + 0·5% North African Rock Phosphate D_1

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·046	10·0
90	0·851	0·055	31·3	5·6	0·057	9·7
180	0·780	0·057	37·1	6·8	0·061	9·0
270	0·659	0·058	46·8	7·4	0·064	8·5
360	0·539	0·057	56·5	8·0	0·065	8·0

TABLE XXXIX

200 gm. Soil + 5 gm. Wheat Straw + 1·0% North African Rock Phosphate D_1

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·062	10·0
90	0·837	0·055	32·4	6·0	0·074	9·7
180	0·769	0·057	37·9	7·3	0·082	9·0
270	0·645	0·058	48·0	7·8	0·087	8·4
360	0·539	0·058	56·5	8·3	0·089	8·0

TABLE XL

200 gm. Soil + 5 gm. Wheat Straw + 0·5% North African Rock Phosphate D_2

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·047	10·0
90	0·851	0·055	31·3	5·6	0·057	9·7
180	0·783	0·057	36·8	6·7	0·061	9·2
270	0·659	0·058	46·8	7·2	0·063	8·6
360	0·537	0·059	56·7	8·0	0·065	8·0

TABLE XLI

200 gm. Soil + 5 gm. Wheat Straw + 1·0% North African Rock Phosphate D_2

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·240	0·052	..	3·5	0·062	10·0
90	0·837	0·056	32·4	6·0	0·073	9·7
180	0·772	0·057	37·7	7·2	0·079	9·1
270	0·643	0·058	48·1	7·7	0·083	8·5
360	0·537	0·059	57·0	8·3	0·085	8·0

TABLE XLII

200 gm. Soil + 10 gm. Wheat Straw + 0·5% Raw Bone-Meal

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·066	10·0
90	1·605	0·069	35·0	6·7	0·093	9·5
180	1·386	0·066	43·8	7·4	0·103	8·6
270	1·185	0·064	52·0	7·8	0·109	8·1
360	1·001	0·062	59·3	8·3	0·113	7·6

TABLE XLIII

200 gm. Soil + 10 gm. Wheat Straw + 1·0% Raw Bone-Meal

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·101	10·0
90	1·570	0·068	36·4	7·0	0·138	9·0
180	1·332	0·066	46·0	7·9	0·140	8·5
270	1·123	0·065	54·5	8·7	0·148	7·9
360	0·947	0·063	61·6	9·0	0·153	7·6

TABLE XLIV

200 gm. Soil + 10 gm. Wheat Straw + 0·5% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	1·470	0·074	..	3·5	0·069	10·0
90	1·615	0·069	34·6	6·7	0·091	9·5
180	1·390	0·068	43·7	7·3	0·099	8·7
270	1·190	0·065	51·8	7·5	0·105	8·3
360	1·015	0·063	58·9	8·2	0·108	7·9

TABLE XLV

200 gm. Soil + 10 gm. Wheat Straw + 1·0% North African Rock Phosphate A

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·107	10·0
90	1·575	0·070	36·2	6·8	0·138	9·1
180	1·335	0·068	45·9	7·9	0·148	8·6
270	1·129	0·066	54·3	8·0	0·154	8·7
360	0·955	0·064	61·3	8·9	0·158	7·7

TABLE XLVI

200 gm. Soil + 10 gm. Wheat Straw + 0·5% North African Rock Phosphate B

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·059	10·0
90	1·623	0·068	34·2	6·5	0·075	9·6
180	1·395	0·065	43·5	7·1	0·082	8·9
270	1·197	0·061	51·5	7·5	0·086	8·4
360	1·020	0·058	58·7	8·1	0·088	7·9

TABLE XLVII

200 gm. Soil + 10 gm. Wheat Straw + 1·0% North African Rock Phosphate B

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·088	10·0
90	1·580	0·068	36·0	6·7	0·111	9·4
180	1·346	0·065	45·5	7·6	0·120	8·8
270	1·140	0·062	53·8	8·3	0·125	8·3
360	0·966	0·059	60·8	8·8	0·129	7·9

TABLE XLVIII

200 gm. Soil + 10 gm. Wheat Straw + 0·5% North African Rock Phosphate C

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·065	10·0
90	1·620	0·069	34·4	6·5	0·082	9·6
180	1·395	0·065	43·5	7·1	0·089	8·8
270	1·193	0·062	51·3	7·5	0·094	8·4
360	1·016	0·059	58·8	8·1	0·097	7·9

TABLE XLIX

200 gm. Soil + 10 gm. Wheat Straw + 1·0% North African Rock Phosphate C

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·098	10·0
90	1·580	0·067	36·0	6·8	0·109	9·2
180	1·341	0·065	45·7	7·6	0·119	8·7
270	1·136	0·062	54·0	8·5	0·125	8·2
360	0·965	0·060	60·9	8·8	0·129	7·8

TABLE L

200 gm. Soil + 10 gm. Wheat Straw + 0·5% North African Rock Phosphate D_1

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·046	10·0
90	1·628	0·066	34·0	6·5	0·059	9·6
180	1·400	0·064	43·3	7·0	0·064	8·9
270	1·200	0·061	51·4	7·5	0·066	8·5
360	1·020	0·057	58·7	7·9	0·068	8·2

TABLE LI

200 gm. Soil + 10 gm. Wheat Straw + 1·0% North African Rock Phosphate D_1

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·061	10·0
90	1·592	0·066	35·5	6·5	0·076	9·4
180	1·356	0·063	45·0	7·4	0·085	8·8
270	1·155	0·061	53·2	7·3	0·091	8·4
360	0·969	0·058	60·7	8·7	0·095	7·9

TABLE LII

200 gm. Soil + 10 gm. Wheat Straw + 0·5% North African Rock Phosphate D_2

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·047	10·0
90	1·625	0·066	34·1	6·5	0·059	9·7
180	1·400	0·064	43·3	7·0	0·064	9·0
270	1·197	0·061	51·5	7·3	0·066	8·5
360	1·022	0·059	58·6	7·9	0·068	8·3

TABLE LIII

200 gm. Soil + 10 gm. Wheat Straw + 1·0% North African Rock Phosphate D_2

Period of exposure in days	Total carbon %	Total nitrogen %	Carbon oxidised %	Ex-Ca in m.e. %	Available P_2O_5 %	pH
0	2·470	0·074	..	3·5	0·062	10·0
90	1·586	0·068	35·7	6·4	0·076	9·5
180	1·350	0·065	45·3	7·4	0·082	8·9
270	1·148	0·062	53·5	8·1	0·085	8·5
360	0·968	0·060	60·8	8·7	0·087	7·9

Discussion

A comparison of these results with those of Mitra, Ghosh and Singh[35] clearly shows that carbonaceous materials in the form of sun-hemp, spent wash and wheat straw are oxidised more quickly when added to alkali soil in conjunction with different phosphatic materials than in the absence of such phosphates.

The carbon of alkali soil (in per cent.) oxidized after treatment with various mixtures of organic matters and phosphatic amendments and $CaCO_3$ as shown in Tables LIV–LVI.

Carbon of soil (in %) oxidised in 360 days

Table LIV

Amendments	200 gm. soil + 13 gm. sun-hemp		200 gm. soil + 26 gm. sun-hemp	
	Amendment		Amendment	
	1%	2%	1%	2%
Calcium carbonate ..	1·428	1·482	2·769	2·985
Basic slag (Tata) ..	1·330	1·428	2·615	2·886
Bihar rock phosphate	1·289	1·339	2·436	2·436

Table LV

Amendments	200 gm. soil + 9·2 gm. spent wash		200 gm. soil + 20·6 gm. spent wash	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
North African rock phosphate A	0·786	0·795	1·554	1·544
Trichinopoly rock phosphate	0·748	0·780	1·568	1·563
Basic slag (Tata) ..	0·798	0·795	1·553	1·573

TABLE LVI

Amendments	200 gm. soil + 5 gm. wheat straw		200 gm. soil + 10 gm. wheat straw	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
Bone-meal ..	0·728	0·737	1·469	1·523
North African rock phosphate:				
A ..	0·707	0·719	1·455	1·515
B ..	0·705	0·703	1·450	1·504
C ..	0·705	0·712	1·454	1·503
D_1 ..	0·701	0·701	1·270	1·501
D_2 ..	0·703	0·703	1·448	1·502

It is well known that alkalinity favours oxidation and that alkalinity of phosphatic amendments depend upon the percentage of lime contained in them. A perusal of Table V will show that lime content (hence alkalinity) of phosphatic amendments used are in the following order:

Basic slag (Tata) > Raw bone-meal > Trichinopoly rock phosphate > North African rock phosphate B > Bihar rock phosphate > North African rock phosphate A > North African rock phosphate C > North African rock phosphate D_1 > North African rock phosphate D_2.

The alkalinity of $CaCO_3$ is certainly much more than that of any of these amendments. The amendments may be divided into two distinct groups: one group consisting of $CaCO_3$, basic slag and raw bone-meal, which have a much greater alkalinity than the other group consisting of the rock phosphates which have nearly the same degree of alkalinity.

It is clear from Table LIV that efficiency of oxidation of sun-hemp in presence of alkali soil and different amendments follow the same order as the alkalinity of the amendments namely:

Calcium carbonate > Basic slag > Bihar rock phosphate.

In case of spent wash, basic slag which is much more alkaline than rock phosphate oxidises more carbon than rock phosphates (Table LV). The

order of oxidation by Trichinopoly rock phosphate and North African rock phosphate A (which have practically the same degree of alkalinity) of spent wash varies with the dose of amendment and concentration of spent wash in a complicated manner. The oxidation is not proportional to alkalinity of rock phosphates. Hence it can be assumed that other factors besides alkalinity of rock phosphates, are responsible for oxidation in this case.

It is obvious from Table LVI that bone-meal which is much more alkaline than any of the rock phosphates has a much greater oxidising power in presence of wheat straw than any of the rock phosphates. The efficiency of oxidation by different rock phosphates, however, do not follow the same order as their alkalinity. This may be due to other factors, besides alkalinity, as postulated in the above paragraph.

It is evident from Tables LIV–LVI that greater oxidation takes place at the same level of organic matter by a greater concentration of the amendment, which can easily be explained by the production of greater alkalinity at higher concentration of the amendment. Moreover it is found that more carbon is oxidised from system at a higher level of carbon than at a lower level in presence of the same amount of the amendment. Pieters[36] has obtained similar increase of oxidation of carbon of organic matter by phosphates.

Nitrogen fixation was observed in some cases (Tables XVIII–XLI), but in others nitrogen loss was observed (Tables VI–XVII and XLII–LII). Nitrogen fixation was only observed in those cases where the initial nitrogen of the system was low and in the cases where the initial nitrogen was high, there was a definite nitrogen loss. Similar results on nitrogen fixation and loss were observed on the application of organic matter alone to alkali soil.[35] Mitra and Shanker[37] have reported nitrogen gain in alkali soils under similar conditions. But in all cases reported by them, the initial nitrogen of the system was very low, which accounted for nitrogen gain. Even they observed that in some cases, there was nitrogen loss after the nitrogen had attained a sufficiently high level by fixation.

A comparison of Tables VIII–XI and XIV–XVII with the results of Mitra, Ghosh and Shankar[35] show that phosphatic amendments checks the nitrogen loss from alkali soils when sun-hemp is applied to it. On the other hand a persual of Tables XLII–LIII with the result of Mitra, Ghosh and Shankar[35] shows that phosphatic amendments have no marked influence on the checking of nitrogen loss from alkali soils on the application of wheat straw. They rather accelerate the nitrogen loss.

The stabilisation of nitrogen by the action of phosphates has been explained by Dhar[27] as follows:

"The proteins that are present in the soil humus are likely to be stabilised by the formation of nucleo-proteins, phospho-proteins, etc., with the combination of proteins and phosphates or other substances."

Moreover, the organic matter present in the soil or added to it undergoes slow oxidation in the soil aided by sunlight and presence of alkali. Under ordinary conditions, the proteins undergo ammonification and nitrification as shown below:

$$\text{Proteins} \rightarrow \text{amino acids} \xrightarrow{O_2} NH_4 \text{ compounds} \xrightarrow{O_2} \text{nitrites} \xrightarrow{O_2} \text{nitrates.}$$

In this series of reactions, an unstable substance NH_4NO_2 is formed, which decomposes resulting in nitrogen loss.

$$NH_4NO_2 = N_2 + 2\ H_2O + 718\ \text{K.Cal.}$$

Dhar emphasises that in presence of phosphates in the system, more or less stable phospho-proteins are formed by the combination of proteins and phosphorus compounds which resist nitrification and ammonification as a result of which nitrogen loss is checked.

In this connection the following lines from Russell[38] is of interest:

"It is not known in what form phytin and nucleic acid occur in the organic matter, though presumably, most of the nucleic acid must be in nucleo-proteins, nor in which humic fractions they are concentrated. They must be protected in some way from the soil enzymes as they are readily dephosphorylated if mixed with the soil, *i.e.*, they must have their phosphate groups split off as inorganic orthophosphate anions. This is also shown independently by the fact that these organic phosphates can only be extracted in good yield from the humic material if it is subjected to fairly drastic pretreatment". Thus the compounds of protenins with phosphoric acid, *viz.*, nucleo-proteins and phosphoproteins are more stable than proteins alone.

The results recorded in Tables LVII–LIX show that exchangeable calcium of the systems greatly increases when $CaCO_3$ and different phosphatic amendments are added to alkali soils in presence of different organic matters.

Increase of Ex-Ca (in m.e. %) after 360 days

TABLE LVII

Amendments	200 gm. soil + 13 gm. sun-hemp		200 gm. soil + 26 gm. sun-hemp	
	Amendment		Amendment	
	1%	2%	1%	2%
Calcium carbonate ..	3·1	4·3	3·6	5·1
Basic slag (Tata) ..	3·5	4·9	4·0	5·1
Bihar rock phosphate ..	4·3	5·2	5·0	6·0

TABLE LVIII

Amendments	200 gm. soil + 9·2 gm. spent wash		200 gm. soil + 20·6 gm. spent wash	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
North African rock phosphate A	4·8	4·9	4·9	5·0
Trichinopoly rock phosphate	4·4	4·5	4·9	4·9
Bihar rock phosphate ..	4·3	4·7	4·6	4·9

TABLE LIX

Amendments	200 gm. soil + 5 gm. wheat straw		200 gm. soil + 10 gm. wheat straw	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
Bone-meal	4·8	5·2	4·8	5·5
North African rock phosphate:				
A ..	4·7	5·0	4·7	5·4
B ..	4·6	4·9	4·6	5·3
C ..	4·6	4·8	4·6	5·3
D_1 ..	4·5	4·8	4·4	5·2
D_2 ..	4·5	4·8	4·4	5·2

The increase in exchangeable calcium observed on the addition of phosphatic amendments along with organic matter is much more than on the addition of organic matter alone. In this case, carbonic and other weak acids produced by the decomposition of organic matter and small amount of nitric acid produced by the nitrification of organic compounds dissolves not only the native soil phosphate from the alkali soil, but also dissolves phosphates from the phosphatic amendments. Soluble calcium ions thus brought into solution replaces sodium ions from the exchange complex of the soil, thereby increasing the exchangeable calcium status of the system.

When $CaCO_3$ is used as an amedment, the following reactions take place:

$$CaCO_3 + H_2CO_3 = Ca(HCO_3)_2$$

$$CaCO_3 + HNO_3 = Ca(NO_3)_2 + H_2CO_3$$

The soluble $Ca(HCO_3)_2$ and $Ca(NO_3)_2$ furnishes Ca^{++} for the improvement of exchangeable calcium status of alkali soils.

The chief phosphatic constituents of rock phosphates carbonatoapatite $[Ca_3(PO_4)_2]_3.CaCO_3$ and fluorapatite $[Ca_3(PO_4)_2]_3.CaF_2$ react with carbonic and nitric acids produced in the alkali soil in the following manner:

$$[Ca_3(PO_4)_2]_3.CaCO_3 + 6\,H_2CO_3 = 3\,Ca(H_2PO_4)_2 + 7\,CaCO_3$$

$$[Ca_3(PO_4)_2]_3.CaF_2 + 7\,H_2CO_3 = 3\,Ca(H_2PO_4)_2 + 7\,CaCO_3 + 2\,HF$$

$$[Ca_3(PO_4)_2]_3 \cdot CaCO_3 + 14\,HNO_3 = 3\,Ca(H_2PO_4)_2 + 7\,Ca(NO_3)_2 + H_2CO_3$$

$$[Ca_3(PO_4)_2]_3.CaF_2 + 14HNO_3 = 3Ca(H_2PO_4)_2 + 7Ca(NO_3)_2 + 2HF$$

The soluble $Ca(H_2PO_4)_2$ and $Ca(NO_3)_2$ produced supplies Ca^{++} for the improvement of exchangeable ealcium status of alkali soils.

Bone is made up largely of calcium phosphate scattered through an organic matrix made up largely of fatty substances containing small amounts of nitrogenous compounds. Hendricks and Hill[39] have presented evidence which indicates that the phosphate found in bones may be a hydrated tricalcium phosphate type of compound containing sodium, magnesium and carbonate as essential constituents. Apparently it is an apatite whose fluorine has been replaced by magnesium and sodium. Klemet[40] showed that while the apatitelike unit structure of tricalcium phosphate hydrate is $(Ca_9)(PO_4)_6(H_2O)_2$ that of human bones is $(Ca_{8\cdot50}Mg_{0\cdot25}Na_{0\cdot19})\,[(PO_4)_{5\cdot07}\,(CO_3)_{1\cdot24}]\,(H_2O)_{2\cdot0}$. It is now generally

accepted that the phosphate compound of bone is carbonatoapatite $[Ca_3 (PO_4)_2]_3 \cdot CaCO_3$, which reacts with H_2CO_3 and HNO_3 as postulated previously producing soluble $Ca (H_2PO_4)_2$ and $Ca (NO_3)_2$.

The most commonly accepted formula for the phosphatic compound found in basic slag is a double silicate and phosphate of lime $[(CaO)_5 \cdot P_2O_5 SiO_2]$, but some chemists consider it a tetracalcium phosphate $(Ca_4P_2O_9)$, while others believe it to be a basic siliconoxyapatite. Lyon and Buckman[41] have suggested that the following reaction takes place on the application of basic slag to soil:

$$(CaO_5 \cdot P_2O_5 \cdot SiO_2 + 8\ CO_2 + 6\ H_2O = Ca (H_2PO_4)_2 + 4\ Ca (HCO_3)_2 + SiO_2.$$

The soluble $Ca (H_2PO_4)_2$ and $Ca (HCO_3)_2$ are effective in the increase of exchangeable calcium.

It has been observed that with increasing dose of amendment at a constant organic matter level and with increasing level of organic matter at the same dose of amendment, the exchangeable calcium status of the system increases. At a higher dose of amendment, there is a greater oxidation of organic matter (due to greater alkalinity) than at a lower dose. Consequently more acids are produced at a higher dose of amendment than at a lower dose at a constant level of organic matter, leading to a greater increase in exchangeable calcium in the former case than in the latter. At a higher concentration of organic matter more CO_2 is produced than at a lower concentration and hence more phosphates are brought into solution at a higher organic matter concentration than at a lower concentration leading to a greater increase of exchangeable calcium in the former case than in the latter case.

The amount of lowering of pH on the addition of phosphatic amendments along with organic matter to alkali soil is shown in Tables LX–LXII.

pH lowered after 360 *days*

TABLE LX

Amendments	200 gm. soil + 13 gm. sun-hemp		200 gm. soil + 26 gm. sun-hemp	
	Amendment		Amendment	
	1%	2%	1%	2%
Calcium carbonate ..	1·5	1·4	1·4	1·4
Basic slag (Tata) ..	1·5	1·5	1·7	1·6
Bihar rock phosphate	1·7	2·0	1·8	2·2

TABLE LXI

Amendments	200 gm. soil + 9·2 gm. spent wash		200 gm. soil + 20·6 gm. spent wash	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
North African rock phosphate A	2·4	2·5	2·5	2·5
Trichinopoly rock phosphate	2·2	2·2	2·3	2·3
Basic slag (Tata) ..	1·6	1·8	1·8	2·0

TABLE LXII

Amendments	200 gm. soil + 5 gm. wheat straw		200 gm. soil + 10 gm. wheat straw	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
Bone-meal ..	2·3	2·4	2·4	2·4
North African rock phosphate:				
A ..	2·2	2·2	2·1	2·3
B ..	2·1	2·1	2·1	2·1
C ..	2·2	2·2	2·1	2·2
D_1 ..	2·0	2·0	1·8	2·1
D_2 ..	2·0	2·0	1·7	2·1

As the exchangeable calcium of a system varies inversely with its pH, hence when the former increases the latter decreases practically in the same proportion. Hence, whatever has been said for the increase in the exchangeable calcium of the system is also responsible for the decrease in the pH value.

In all cases, the incorporation of phosphatic amendments with organic materials lowers the pH values to a greater extent than when organic matter alone is added to alkali soil.

The oxidation of organic materials like sun-hemp, spent wash and wheat straw enhances the availability of the phosphates of the system (Tables LXIII–LXV).

Increase of available P_2O_5 (in %) after 360 days

TABLE LXIII

Amendments	200 gm. soil + 13 gm. sun-hemp		200 gm. soil + 26 gm. sun-hemp	
	Amendment		Amendment	
	1%	2%	1%	2%
Calcium carbonate ..	0·029	0·041	0·035	0·055
Basic slag (Tata) ..	0·032	0·044	0·043	0·056
Bihar rock phosphate ..	0·032	0·039	0·052	0·060

TABLE LXIV

Amendments	200 gm. soil + 9·2 gm. spent wash		200 gm. soil + 20·6 gm. spent wash	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
North African rock phosphate A	0·051	0·043	0·061	0·056
Trichinopoly rock phosphate	0·053	0·057	0·067	0·064
Basic slag (Tata) ..	0·0314	0·051	0·047	0·062

TABLE LXV

Amendments	200 gm. soil + 5 gm. wheat straw		200 gm. soil + 10 gm. wheat straw	
	Amendment		Amendment	
	0·5%	1·0%	0·5%	1·0%
Bone-meal ..	0·041	0·049	0·047	0·052
North African rock phosphate:				
A ..	0·035	0·044	0·039	0·051
B ..	0·027	0·033	0·029	0·041
C ..	0·030	0·030	0·032	0·031
D_1 ..	0·019	0·027	0·022	0·034
D_2 ..	0·018	0·023	0·021	0·025

The soluble calcium compound $Ca(H_2PO_4)_2$ formed in the system (as already discussed) increases the availability of PO_4 in the system.

It has been observed that increase of available phosphate varies directly with the rate of oxidation of carbon. There is always a greater increase in available phosphate in spent wash treated soil. This may be ascribed to the fact that along with carbonic acid, other organic acids like acetic, propionic, lactic, etc., are produced during the fermentation of sugars present in spent wash. They jointly dissolve more phosphate, as a result of which greater increases in available phosphate is obtained in this treatment.

It is observed that a much greater increase of available phosphate is obtained by the incorporation of phosphatic amendments along with organic matter to alkali soil than when organic matter alone is incorporated. This enhanced availability can be ascribed to the soluble phosphates obtained from the phosphatic amendments.

Native soil phosphate, phosphatic amendments and soil organic matter are the three sources of supply of available phosphates. It is difficult to say as to what proportion of the increased available phosphates comes from each of the above three sources.

The following observation of Dhar[42] is of interest in this connection:

"The third dissociation constant of phosphoric acid is smaller than the first and second dissociation constants of carbonic acid and hence carbonic acid converts tricalcium phosphate into dicalcium phosphate, which is more soluble than tricalcium phosphate. Thus, the availability of phosphates is increased in the soil richer in carbonic acid, obtained from the addition of organic substances. Similarly nitrous acid and nitric acids formed in the ammonification and nitrification of proteins make phosphate more readily available. Thus, phosphates are more useful in presence of organic substances like form manure, straw, etc., undergoing slow oxidation".

Sieling and Struther[43] advanced organic acid theory for increasing the availability of phosphate during decomposition of organic matter in the soil. They claim that anions like citrate, oxalate, tartrate, malate and malonate are effective in replacing phosphates and these are produced in abundance during the decomposition of organic matter by micro-organisms. They have obtained considerable evidence that pectic acid and galactouronic acids act as effective complexing agent *in vitro*.

Considerable evidence has accumulated in recent years on the value of phosphatic amendments in combination with organic matter in the improvement of alkaline soils. McGeorge[4] found that organic matter was specially valuable in increasing the value of phosphatic manures on alkaline calcareous soils. Rhoades[44] noted that increases in soluble phosphate take place on the application of crop residues and manure along with rock phosphates to alkaline soils.

Singh and Nijhawan[45] observed that application of farmyard manure considerably increased the otherwise depressed uptake of phosphorus by plants on an alkaline soil. After application of $CaCl_2$ the availability of phosphorus in presence of farmyard manure was further increased. Mitra and Shanker[46] observed that in presence of Assam coal and lignite, rock phosphate and basic slag increased available phosphate and decreased the pH of alkali soil from Soraon (Allahabad).

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part I. Effect of Composition of the Media on Yeast Growth

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SINCE the publication of the celebrated memoir " Etudes sur la biere " by Pasteur, the yeasts have assumed an ever-increasing importance in the manifold branches of industries and dietaries. Of late, however, the attention of the scientists has been focussed on the use of yeasts as supplementary food. Yeasts form an efficient protein manufactured from the cheapest raw materials. Two of the major defects in many of the national diets are small provisions of certain vitamins of B-complex group and an insufficiency of the biologically superior protein. Both these deficiencies could be minimised by using yeast as supplementary food. Apart from these, yeast is rich in certain minerals. The technical committee on nutrition of the " League of Nations " recommended the inclusion of yeast in tropical diets.

The chief difficulty in the commercial production of yeast lies in its low yield. In his classical experiments, Pasteur added 2·622 grams of yeast to a solution of 100 grams of sugar in 750 c.c. water and obtained 2·965 grams of yeast after the lapse of 20 days. Thus it is clear that the yield of yeast is small as compared to the amount of sugar used.

A new variety of yeast *Dhar yeast* which develops very vigorously after a short period of incubation has been isolated from the palm-toddy of Allahabad by Dhar and Bahadur.[1] The growth of this yeast has been studied and the yield has been compared with those obtained with *Saccharomyces pastorianus, Saccharomyces cerevisiæ, Torula yeast* and *Saccharomyces uvarum*. Morphological examination shows (Table I) that this yeast belongs to the genus *Pichia* but as the properties of the strain differs in many respects from the properties of various yeasts described under the same genus, the strain is designated as *Dhar yeast*.

EXPERIMENTAL

200 c.c. of standard media of the following composition was taken in a number of 250 c.c. conical flasks.

Potassium dihydrogen phosphate	..	0·380 gm.
Ammonium sulphate	,, ,,	1·320 gm.

TABLE

Morphological Characteristics

Mark	Appearance and size of cells in liquid wort after 24 hours	Film formation and size of cells in film	Spore formation		Slide culture
			On filter-paper (=gypsum block)	On Gorodkowa agar	
1	Round and egg-shaped, 7–9 μ	–	After 2 days at 25° C. Spores as top yeast spores with septæ	..	No formation of Pseudomycelium
2	Round, 4–8 μ, mostly of *ca.* 4 μ	–	After 14 days at 25° C. No Spores	7 days at 25° C. Few	do.
3	Round, and oval. Round, 5–8 μ. Oval, 13 μ long, *ca.* 5 μ wide	–	2 days at 25° C. Spore formation as in pastorianus yeast	..	Tree like formation of Pseudomycelium
4	Round and oval. Round, 5–7 μ. Oval, 13 μ long, *ca.* 5 μ wide	–	14 days at 25° C. No spores	3 days at 25° C. Only few (2 round spores per ascus)	As No. 3
5	Round and oval. 7–9 μ	Dry film creeping up side of glass. Cell length 5–7 μ	7 days at 25° C. 2 round spores per ascus with oil droplets in spores	..	do.

I

of Yeasts

Fermentation of sugars	Assimilation of sugars	Assimilation of potassium nitrate	Growth using Ethanol as sole source of carbon	Identification of micro-organisms	Remarks
Glucose + Galactose + Sucrose + Maltose + Lactose − Raffinose −	Glucose + Galactose + Sucrose + Maltose + Lactose −	−	−	*Saccharomyces cerevisiæ*	..
Glucose + Galactose (+) Sucrose + Maltose (+) Lactose − Raffinose −	Glucose + Galactose + Sucrose + Maltose (+) Lactose −	+	+	A spore forming *Torula yeast*	The determination is not certain
Glucose + Galactose + Sucrose + Maltose + Lactose − Raffinose +	Glucose + Galactose − Sucrose + Maltose + Lactose −	−	−	*Saccharomyces pastorianus*	Diverge from the normal type by fermenting Galactose
Glucose + Galactose + Sucrose + Maltose + Lactose − Raffinose +	Glucose + Galactose + Sucrose + Maltose + Lactose −	+	−	*Saccharomyces uvarum*	..
Glucose + Galactose − Sucrose + Maltose − Lactose − Raffinose −	Glucose + Galactose (+) or − Sucrose + Maltose (+) or − Lactose (+) or −	−	+	Pichia variety	Probably Pichia Polymorpha. Referred all through as *Dhar yeast*

(−) Denotes negative; (+) Denotes very feeble.

Potassium sulphate	0·100 gm.
Calcium carbonate	0·025 gm.
Sodium chloride	0·006 gm.
Magnesium sulphate	0·044 gm.
Zinc sulphate	0·025 gm.
Ferrus sulphate	0·003 gm.
Glucose	10·000 gm.
Distilled water to make	200 c.c.

The pH of the medium was adjusted to 4·5 by using dilute HCl. The flasks were plugged with sterilized cotton wool and were then sterilized at 10 lb. pressure for 30 minutes. The flasks were inoculated with different yeasts and the flasks were allowed to stand for the growth of yeast. After definite time interval, the yeasts were filtered and the weight of dry yeast was determined.

RESULTS

Effect of Different Energy Materials

Media were prepared as described earlier. In each case, different energy materials equivalent to 10 grams of glucose were added.

Total Volume	200 c.c.
pH	4·5
Time	20 days
Temperature	25° C.

TABLE II

Dhar yeast

Energy material	Amount added (Glucose equivalent) (gm.)	Amount left (gm.)	Amount utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Glucose ..	10·0000	3·5148	6·4852	1·4274	22·01
Galactose ..	10·0000	..	..	Nil	..
Maltose ..	10·0000	..	..	Nil	..
Lactose ..	10·0000	..	..	Nil	..
Sucrose ..	10·0000	4·4212	5·5788	1·1871	21·28

TABLE III

S. pastorianus

Energy material	Amount added (Glucose equivalent) (gm.)	Amount left (gm.)	Amount utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Glucose ..	10·0000	Nil	10·0000	0·1642	1·64
Galactose ..	10·0000	..	..	Nil	..
Maltose ..	10·0000	0·4822	9·5178	0·2120	2·23
Lactose ..	10·0000	..	..	Nil	..
Sucrose ..	10·0000	Nil	10·0000	0·1870	1·87

TABLE IV

S. cerevisiæ

Energy material	Amount added (Glucose equivalent) (gm.)	Amount left (gm.)	Amount utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Glucose ..	10·0000	Nil	10·0000	0·1094	1·09
Galactose ..	10·0000	8·1005	1·8995	0·0430	2·27
Maltose ..	10·0000	0·4335	9·5665	0·1792	1·87
Lactose ..	10·0000	..	..	Nil	..
Sucrose ..	10·0000	Nil	10·0000	0·1280	1·28

TABLE V

Torula yeast

Energy material	Amount added (Glucose equivalent) (gm.)	Amount left (gm.)	Amount utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Glucose ..	10·0000	4·2500	5·7500	0·1276	2·22
Galactose	10·0000	7·9239	2·0761	0·0538	2·59
Maltose ..	10·0000	..	..	Nil	..
Lactose ..	10·0000	..	..	Nil	..
Sucrose ..	10·0000	5·5061	4·4939	0·1322	2·94

TABLE VI

S. uvarum

Energy material	Amount added (Glucose equivalent) (gm.)	Amount left (gm.)	Amount utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Glucose ..	10·0000	Nil	10·0000	0·1406	1·41
Galactose ..	10·0000	8·2609	1·7391	0·0502	2·88
Maltose ..	10·0000	4·3750	5·6250	0·1784	3·17
Lactose ..	10·0000	..	..	Nil	..
Sucrose ..	10·0000	Nil	10·0000	0·1792	1·78

Effect of Different Concentrations of Glucose

Composition of the media were maintained the same with the only difference that different amounts of glucose were added.

Total Volume	200 c.c.
pH	4·5
Time	20 days
Temperature	25° C.

TABLE VII

Dhar yeast

Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
2·0000	Nil	2·0000	0·5470	27·35
5·0000	0·3366	4·6634	1·3036	27·95
10·0000	3·5186	6·4814	1·3598	20·98
15·0000	3·7005	11·2995	1·5464	13·69
20·0000	6·7179	13·2821	1·6848	12·68

TABLE VIII

S. pastorianus

Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
2·0000	Nil	2·0000	0·0762	3·81
5·0000	Nil	5·0000	0·1531	3·06
10·0000	Nil	10·0000	0·1942	1·94
15·0000	0·5107	14·4893	0·2064	1·42
20·0000	1·5293	18·4707	0·2130	1·15

TABLE IX

S. cerevisiæ

Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
2·0000	Nil	2·0000	0·0878	4·39
5·0000	Nil	5·0000	0·1778	3·56
10·0000	Nil	10·0000	0·1842	1·84
15·0000	0·7344	14·2655	0·2042	1·43
20·0000	0·8924	19·1076	0·2330	1·22

TABLE X

Torula yeast

Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
2·0000	Nil	2·0000	0·0522	2·61
5·0000	1·7083	3·2917	0·0948	2·88
10·0000	6·3459	3·6541	0·1118	3·06
15·0000	9·9491	5·0509	0·1258	2·48
20·0000	14·0430	5·9570	0·1308	2·19

TABLE XI

S. uvarum

Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
2·0000	Nil	2·0000	0·0748	3·74
5·0000	Nil	5·0000	0·1452	2·90
10·0000	Nil	10·0000	0·1676	1·68
15·0000	Nil	15·0000	0·2041	1·36
20·0000	1·0217	18·9783	0·2218	1·17

Effect of Different Phosphates

Standard media containing glucose was used in all experiments with the only difference that different phosphates were used. The amount of phosphate added was so adjusted that the percentage of P_2O_5 in the different media remained constant. The media were filtered whenever necessary.

Total Volume .. .. .. 200 c.c.

pH .. .. 4·5

Time .. .. 20 days

Temperature .. .. .. 25° C.

TABLE XII

Dhar yeast

Different phosphates	Phosphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
$CaHPO_4$ ..	0·3800	10·0000	5·1862	4·8138	1·0263	21·32
$CaH_4(PO_4)_2.H_2O$	0·3520	10·0000	5·2468	4·7532	1·2229	25·73
$Ca_3(PO_4)_2$ ..	0·4331	10·0000	7·6746	2·3254	0·8222	18·12
$Na_2HPO_4.H_2O$ ..	0·4470	10·0000	4·6306	5·3694	1·2339	22·98
KH_2PO_4 ..	0·3800	10·0000	4·7588	5·2412	1·2904	24·62

TABLE XIII

S. pastorianus

Different phosphates	Phosphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
$CaHPO_4$ ..	0·3800	10·0000	Nil	10·0000	0·1352	1·35
$CaH_4(PO_4)_2.H_2O$	0·3520	10·0000	Nil	10·0000	0·1408	1·41
$Ca_3(PO_4)_2$ ..	0·4331	10·0000	Nil	10·0000	0·1056	1·06
$Na_2HPO_4.H_2O$ ..	0·4470	10·0000	1·0641	8·9359	0·1058	1·18
KH_2PO_4 ..	0·3800	10·0000	Nil	10·0000	0·1516	1·52

TABLE XIV

S. cerevisiæ

Different phosphates	Phosphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
$CaHPO_4$ ..	0·3800	10·0000	1·0116	8·9884	0·1236	1·37
$CaH_4(PO_4)_2.H_2O$	0·3520	10·0000	Nil	10·0000	0·1634	1·63
$Ca_3(PO_4)_2$ ..	0·4331	10·0000	3·7600	6·2400	0·0936	1·50
$Na_2HPO_4.H_2O$ ..	0·4470	10·0000	2·2226	7·7774	0·1228	1·58
KH_2PO_4 ..	0·3800	10·0000	1·0875	8·9125	0·1762	1·98

TABLE XV

Torula yeast

Different phosphates	Phosphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
$CaHPO_4$ ..	0·3800	10·0000	4·9155	5·0845	0·8880	1·74
$CaH_4(PO_4)_2.H_2O$	0·3520	10·0000	4·8807	5·1193	0·0901	1·76
$Ca_3(PO_4)_2$ ..	0·4331	10·0000	6·4575	3·5424	0·0584	1·65
$Na_2HPO_4.H_2O$ ..	0·4470	10·0000	5·9243	4·0757	0·0644	1·58
KH_2PO_4 ..	0·3800	10·0000	3·9198	6·0802	0·1201	1·99

TABLE XVI

S. uvarum

Different phosphates	Phosphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
$CaHPO_4$ ..	0·3800	10·0000	Nil	10·0000	0·1080	1·08
$CaH_4(PO_4)_2.H_2O$	0·3520	10·0000	Nil	10·0000	0·1342	1·34
$Ca_3(PO_4)_2$ ..	0·4331	10·0000	Nil	10·0000	0·0934	0·09
$Na_2HPO_4.H_2O$ ..	0·4470	10·0000	Nil	10·0000	0·0682	0·68
KH_2PO_4 ..	0·3800	10·0000	Nil	10·0000	0·1364	1·36

Effect of Different Concentrations of Potassium Dihydrogen Phosphate

Standard media containing glucose was used in all experiments with the only difference that different concentrations of potassium dihydrogen phosphate KH_2PO_4 was used in different experiments.

Total Volume	200 c.c.
pH	4·5
Time	20 days
Temperature	25° C.

TABLE XVII

Dhar yeast

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3600	10·0000	3·1716	6·8284	1·3691	20·05
0·6800	10·0000	3·9409	6·0591	1·1894	19·63
0·3400	10·0000	4·9186	5·0814	1·2612	24·82
0·1700	10·0000	4·7929	5·2071	0·8914	17·12
0·0850	10·0000	4·1479	4·8521	0·6133	12·64

TABLE XVIII

S. pastorianus

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3600	10·0000	Nil	10·0000	0·2238	2·23
0·6800	10·0000	Nil	10·0000	0·2224	2·22
0·3400	10·0000	Nil	10·0000	0·2091	2·09
0·1700	10·0000	3·4839	6·5168	0·1322	2·03
0·0850	10·0000	3·7861	6·2139	0·1244	2·01

TABLE XIX

S. cerevisiæ

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3600	10·0000	Nil	10·0000	0·1682	1·68
0·6800	10·0000	0·0642	9·9358	0·1340	1·48
0·3400	10·0000	2·7967	7·2033	0·1312	1·82
0·1700	10·0000	4·4003	5·5997	0·0940	1·64
0·0850	10·0000	5·7945	4·2055	0·0614	1·46

TABLE XX

Torula yeast

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3600	10·0000	3·3653	6·6347	0·0804	1·21
0·6800	10·0000	4·8142	5·1858	0·0784	1·52
0·3400	10·0000	3·9677	6·0323	0·1122	1·86
0·1700	10·0000	5·6975	4·3025	0·0512	1·19
0·0850	10·0000	6·3405	3·6595	0·0413	1·13

TABLE XXI

S. uvarum

KH_2PO_4 added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3600	10·0000	Nil	10·0000	0·1884	1·88
0·6800	10·0000	0·4349	9·5651	0·1502	1·57
0·3400	10·0000	0·6423	9·3577	0·1282	1·37
0·1700	10·0000	5·2727	4·7273	0·0614	1·29
0·0850	10·0000	6·6829	3·3171	0·0408	1·23

Effect of Different Nitrogenous Compounds

Standard media containing glucose was used in all experiments with the only difference that different nitrogenous compounds were used. The amount of nitrogenous compound added was so adjusted that the amount of nitrogen remained the same (0·28 grams) in different media.

Total Volume	200 c.c.
pH	4·5
Time	20 days
Temperature	25° C.

TABLE XXII

Dhar yeast

Nitrogen sources	Nitrogenous compound added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
mmonium sulphate	1·3200	10·0000	5·5788	4·4212	1·1871	26·8
mmonium nitrate	0·8000	10·0000	8·1190	1·8910	0·4164	22·02
mmonium acetate	1·5400	10·0000	7·4334	2·5666	0·5378	20·93
rea	0·6000	10·0000	6·1873	3·8027	0·0586	15·41
mmonium chloride	1·0700	10·0000	6·3825	3·6075	0·8593	23·82

TABLE XXIII

S. pastorianus

Nitrogen sources	Nitrogenous compound added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
mmonium sulphate	1·3200	10·0000	Nil	10·0000	0·1920	1·92
mmonium nitrate	0·8000	10·0000	2·8600	7·1400	0·0766	1·07
mmonium acetate	1·5400	10·0000	Nil	10·0000	0·1818	0·08
rea	0·6000	10·0000	Nil	10·0000	0·3336	3·33
mmonium chloride	1·0700	10·0000	Nil	10·0000	0·1474	1·47

TABLE XXIV

S. cerevisiæ

Nitrogen sources	Nitrogenous compound added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Ammonium sulphate	1·3200	10·0000	0·8861	9·1139	0·1148	1·26
Ammonium nitrate	0·8000	10·0000	2·7032	7·2968	0·0918	1·26
Ammonium acetate	1·5400	10·0000	Nil	10·0000	0·0610	0·61
Urea	0·6000	10·0000	Nil	10·0000	0·2148	2·14
Ammonium chloride	1·0700	10·0000	2·6047	7·3953	0·1134	1·53

TABLE XXV

Torula yeast

Nitrogen sources	Nitrogenous compound added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Ammonium sulphate	1·3200	10·0000	5·5937	4·4063	0·0912	2·02
Ammonium nitrate	0·8000	10·0000	6·0678	3·9322	0·0314	0·79
Ammonium acetate	1·5400	10·0000	6·7041	3·2959	0·0580	1·76
Urea	0·6000	10·0000	5·9076	4·0924	0·0428	1·04
Ammonium chloride	1·0700	10·0000	5·6825	4·3175	0·0960	2·22

TABLE XXVI

S. uvarum

Nitrogen sources	Nitrogenous compound added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
Ammonium sulphate	1·3200	10·0000	Nil	10·0000	0·1342	1·34
Ammonium nitrate	0·8000	10·0000	1·8523	8·1477	0·0526	0·64
Ammonium acetate	1·5400	10·0000	Nil	10·0000	0·0636	0·63
Urea	0·6000	10·0000	Nil	10·0000	0·2400	2·40
Ammonium chloride	1·0700	10·0000	Nil	10·0000	0·1092	1·09

Effect of Different Concentrations of Ammonium Sulphate

Standard media containing glucose was used in all experiments with the only difference that different concentrations of ammonium sulphate was used.

Total Volume .. .. .. 200 c.c.
pH .. .. 4·5
Time .. .. 20 days
Temperature .. .. .. 25° C.

TABLE XXVII

Dhar yeast

Ammonium sulphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3200	10·0000	4·3587	5·6413	1·3212	23·42
0·3300	10·0000	7·5028	2·4972	0·7644	30·61
0·0825	10·0000	9·2704	0·7396	0·1687	22·81
0·0206	10·0000	9·8051	0·1949	0·0402	20·62
0·0051	10·0000	9·9377	0·0623	0·0115	18·54

TABLE XXVIII

S. pastorianus

Ammonium sulphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3200	10·0000	0·5870	9·4130	0·1736	1·84
0·3300	10·0000	1·0421	8·9579	0·1576	1·76
0·0825	10·0000	3·3145	6·6855	0·1016	1·52
0·0206	10·0000	3·7586	6·2414	0·0724	1·16
0·0051	10·0000	4·7429	5·2571	0·0570	1·08

TABLE XXIX

S. cerevisiæ

Ammonium sulphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3200	10·0000	1·0875	8·9125	0·1762	1·98
0·3300	10·0000	2·6024	7·3976	0·1368	1·85
0·0825	10·0000	4·3009	5·6991	0·1170	2·05
0·0206	10·0000	5·0432	4·9568	0·0742	1·48
0·0051	10·0000	5·3798	4·6202	0·0616	1·33

TABLE XXX

Torula yeast

Ammonium sulphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3200	10·0000	3·1198	6·8802	0·0688	1·00
0·3300	10·0000	4·4779	5·5221	0·0776	1·41
0·0825	10·0000	4·5447	5·4553	0·0674	1·23
0·0206	10·0000	3·9341	6·0659	0·0658	1·08
0·0051	10·0000	3·3805	6·6195	0·0564	0·85

TABLE XXXI

S. uvarum

Ammonium sulphate added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose utilised (gm.)	Yeast produced (gm.)	Percentage growth based on sugar consumption
1·3200	10·0000	0·4350	9·5650	0·1712	1·79
0·3300	10·0000	1·9519	8·0481	0·1183	1·47
0·0825	10·0000	3·5407	6·4593	0·0846	1·31
0·0206	10·0000	4·2645	5·7355	0·0694	1·21
0·0051	10·0000	6·7728	3·2271	0·0326	1·01

DISCUSSION

Carbonaceous compounds supply energy required by the living organisms. Almost half the dry weight of the cells consist of carbon. Protoplasm, the cell wall and reserve nutrients stored within the cells are composed of carbon. Yeasts secure energy by oxidising organic compounds.

It can be concluded from our observation that yeasts are selective in the utilisation of carbohydrates. *Dhar yeast* does not grow in galactose, maltose and lactose. Other yeasts also did not grow in all the sugars used. Such specific action of the yeasts have also been observed by other workers. Rao and Krishnamachari[2] have tested *Torula, Candida, Schizesaccharomyces* and *Rhodotorula* for their ability to ferment seven mono-saccharides and two disaccharides. It was observed that these varieties of yeasts were less active biochemically than *Saccharomyces* and that *Rhodotorula* could not ferment any of the carbohydrates employed. Skog and Lindegreen[3] reported the behaviour of 12 strains of *S. cerevisiæ* which did not utilize glucose.

The concentrations of energy materials were found to exert a great influence on the growth efficiency of the yeasts. With the exception of *Torula yeast*, which was found to have an optimum concentration, the growth efficiency in all other cases was found to be inversely related to the concentration of sugar used. In all cases, however, the growth efficiency of *Dhar yeast* was many times greater than other varieties.

Phosphorus compounds play an important role in the chemical transformation of energy materials during yeast growth. Phosphorus appears to participate in almost every step in the anaerobic conversion of glucose into alcohol by the yeast. Harden[4] has shown that phosphorus is required in the enzymatic transformation of glucose into alcohol and carbon dioxide. Phosphorus is utilized only when it is in the form of phosphate, mainly as phosphate ester. Sodium hypophosphite and sodium phosphite cannot be utilized by yeasts.

The different phosphates were found to have different effects on yeast growth. Potassium dihydrogen phosphate was found to give the maximum growth with all the yeasts which showed that potassium ion is also necessary for yeast growth. This conclusion has been supported by the work of Neuberg and Lustig[5] who have shown that some potassium is indispensable for some partial processes of fermentation. It was also observed that sodium does not favour yeast growth. Among the three phosphates of calcium used, the maximum growth was observed with the readily soluble monocalcium phosphate. Except in the case of *Torula yeast*, which required an optimum concentration for growth, in all other cases the cell yield depended upon the concentration of the phosphate.

Nitrogen is an essential element for yeast growth and yeast dry matter contains on an average 8·5% N. In the nutrition of yeasts, ammonium salts play an important role while the nitrates as sources of nitrogen are much less efficient. Some workers believe that yeasts can utilize nitrates efficiently but others like Bokorny[6] disagree with this view. Pirochle[7] in his experiments observed that nitrates do not favour growth because they are reduced to nitrites, which acts as poison. He also reported that if accumulation of nitrite is prevented by aeration or addition of hydrogen peroxide, then nitrates act as efficiently as ammonium salts. Linder and Wust[8] found that urea can serve as source of nitrogen and not of carbon for yeast growth. Bokorny[9] measured the increase in the development of yeast, when nitrogen was taken from urea and concluded that yeasts can utilize nitrogen of urea.

It was observed that urea produced the maximum yield for *Saccharomyces yeasts*. In presence of ammonium acetate, however, lower yields of *Saccharomyces yeasts* were obtained. In case of *Torula* and *Dhar yeasts*, sulphate and chloride of ammonium served as the best source of nitrogen. Ammonium nitrate produced low yields in all cases because nitrate was reduced to nitrite which was toxic for yeast growth. *Dhar yeast* showed a cell yield which was many times higher than other varieties. The yield of

yeasts decreased with decrease of ammonium sulphate concentration. *Torula* and *Dhar yeasts* showed a maximum of growth efficiency at a lower concentration of ammonium sulphate.

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part II. Effect of Sugars, Alcohol and Glycerol on Yeast Growth

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As early as 1889 Laurent[1] observed that acetates, lactates, citrates, tartarates, and succinates and tartaric, malic, succinic, and lactic acids, glycerol and sugars of $C_6H_{12}O_6$ and $C_{12}H_{22}O_{11}$ series, and substances which can be transformed into glucosides such as lecithin, dextrin, asparagine and peptones can be used as energy materials by yeasts. He also found that alcohols, aldehydes, ethers, fatty acids, amides and hydroquinone or cellulose cannot be used by yeasts as sources of carbon. Later on other workers[2, 3] reported that the yeasts can utilize ethyl alcohol as a source of carbon for their growth. Taketomi and coworkers[4] have cultivated aerobically *Baker's yeast*, *Torulopsis utilis* and *Mycotorula japonica*, in a media containing ethyl alcohol as a source of carbon. Rao and Krishnamachari[5] studied the fermentation of twenty-two carbohydrates with ten species of yeasts. We have also used some organic compounds as source of carbon for the growth of *Saccharomyces cerevisiæ*, *Torulopsis utilis*, *Dhar yeast*[6] and *Rhodotorula gracilis*. It is reported[7, 8] that *Dhar yeast* grows abundantly in media containing ethyl alcohol as the only source of carbon.

EXPERIMENTAL

Known volumes of media of the following composition were transferred to 250 c.c. conical flasks:

Substance	Amount in 1,000 c.c. media
Ammonium sulphate	4·0 gm.
Potassium sulphate	4·0 ,,
Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12\ H_2O$)	4·0 ,,
Magnesium sulphate	0·5 ,,
Calcium chloride	0·1 ,,
Ferric ammonium sulphate ..	0·02 mgm.
Zinc (as sulphate)	0·20 ,,
Copper (as sulphate)	0·01 ,,

Substance	Amount in 1,000 c.c. media
Aneurin hydrochloride ..	200 micrograms
Riboflavin	100 ,,
Nicotinic acid	5,000 ,,
p-amino benzoic acid	300 ,,
Pyridoxin	1,000 ,,
Ca-D-pantothenate	500 ,,
D-biotin	6 ,,

Calculated amounts of different sugars were added to each flask, so that in every case the amount of sugar present is equivalent to 5 gm. of glucose. The pH of the media was adjusted to 4·5 by dilute hydrochloric acid, and the volume in each flask was made upto 100 c.c. The flasks were cotton-plugged and autoclaved at 15 lb. pressure for 20 minutes. The flasks were cooled and seeded with 0·05 c.c. of active yeast culture. In cases where ethyl alcohol or glycerol were employed, known amounts of respective material were added to the media either before autoclaving as in the case of glycerol or after autoclaving as in the case of ethyl alcohol. The seeded flasks were incubated at 35° C. for twenty days, after which period the contents of each flask was analysed.

Estimation of the quantity of yeast was done by filtering the contents of the flask through two oven-dried Whatman No. 1 filter-papers, which were previously weighed. The residue was washed with water and afterwards air-dried at 80° C. for 6 hours. The filter-papers along with residue were weighed again. The difference gave the weight of the yeast.

Estimation of glucose was done by the reduction of Fehling solution using 1 per cent. aqueous solution of methylene blue as indicator. In the case of di- and trisaccharides the estimation of glucose was done after hydrolysing the solution by concentrated hydrochloric acid. The hydrolysis of sugar was done by adding 2 c.c. concentrated HCl to the media, and placing the solution over boiling water-bath for half an hour. It was diluted with water and allowed to stand overnight. Estimation of sugar was done after neutralising this solution with alkali.

The yeast was filtered from the medium. The volume of the filtrate was made up to 250 c.c. 100 c.c. of this solution and 50 c.c. of water were added to a 500 c.c. distillation flask. Nearly 100 c.c. of the liquid were distilled off in a pyknometer. The relative density of the distillate was determined by weighing and the amount of alcohol present was calculated from the alcohol tables.

RESULTS

TABLE I

S. cerevisiæ

Energy material	Glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
Glucose ..	5·0000	Nil	5·0000	0·1401	2·8
Sucrose ..	5·0000	Nil	5·0000	0·1584	3·1
Maltose ..	5·0000	2·6482	2·3518	0·0363	1·6
Lactose ..	5·0000	5·0000	Nil	Nil	Nil
Arabinose ..	5·0000	5·0000	Nil	Nil	Nil
Raffinose ..	5·0000	0·6981	4·3016	0·0700	1·6
Galactose ..	5·0000	4·6296	0·3704	0·0576	15·6

TABLE II

T. utilis

Energy material	Glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
Glucose ..	5·0000	Nil	5·0000	0·1522	3·0
Sucrose ..	5·0000	Nil	5·0000	0·1702	3·4
Maltose ..	5·0000	2·6482	2·3518	0·0554	2·0
Lactose ..	5·0000	5·0000	Nil	Nil	Nil
Arabinose ..	5·0000	5·0000	Nil	Nil	Nil
Raffinose ..	5·0000	0·6052	4·3948	0·1250	2·8
Galactose ..	5·0000	4·6296	0·3704	0·0448	12·1

TABLE III

Dhar yeast

Energy material	Glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
Glucose ..	5·0000	1·2500	3·7500	0·5312	14·7
Sucrose ..	5·0000	0·9786	4·0214	0·7694	19·1
Maltose ..	5·0000	2·0161	2·9839	0·0190	0·7
Lactose ..	5·0000	5·0000	Nil	Nil	Nil
Arabinose ..	5·0000	5·0000	Nil	Nil	Nil
Raffinose ..	5·0000	1·0416	3·9584	0·0462	1·2
Galactose ..	5·0000	4·6296	0·3704	0·0399	10·8

TABLE IV

R. gracilis

Energy material	Glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
Glucose ..	5·0000	2·6500	2·3500	0·3804	16·0
Sucrose ..	5·0000	2·5244	2·4756	0·3762	15·2
Maltose ..	5·0000	2·0161	2·9839	0·3816	12·1
Lactose ..	5·0000	5·0000	Nil	Nil	Nil
Arabinose ..	5·0000	4·1682	0·8318	0·2417	29·1
Raffinose ..	5·0000	4·3410	0·5590	0·1892	33·8
Galactose ..	5·0000	3·4728	0·5272	0·2260	42·8

TABLE V

Ethyl alcohol as a source of carbon for Dhar yeast

Ethyl alcohol added (gm.)	Alcohol lost by evaporation (gm.)	Alcohol left (gm.)	Alcohol used (gm.)	Yeast grown (gm.)	Efficiency (%)
0·7890	0·2623	0·3675	0·1592	0·0668	43·7
1·5780	0·4004	0·7613	0·4163	0·1683	40·4
2·3670	0·9546	0·7830	0·6294	0·2224	34·8
3·1560	1·1006	1·0911	0·9643	0·2652	27·5
3·9450	1·2738	1·2680	1·4014	0·3000	20·1

It was also observed that *Dhar yeast* grow well in media containing ethyl alcohol as the chief source of carbon. *S. cerevisiæ* did not grow at all. *T. utilis* and *R. grcilis* could survive in media containing 1 per cent. ethyl alcohol V/V, but the growth was not appreciable.

S. cerevisiæ, *T. utilis* and *Dhar yeast* could grow well in media containing 5% glycerol V/V. The respective yields of yeast from 100 c.c. of media were 0·3182 gm., 0·7360 gm. and 0·2306 gm.

EFFECT OF CONCENTRATION OF GLUCOSE ON YEAST GROWTH

TABLE VI

S. cerevisiæ

Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·1034	10·3
3·0000	Nil	3·0000	0·1160	3·9
5·0000	Nil	5·0000	0·1401	2·8
7·0000	1·8939	5·1061	0·1405	2·8
10·0000	5·1650	4·8350	0·1412	2·9

TABLE VII

T. utilis

Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·1116	11·2
3·0000	Nil	3·0000	0·1338	4·5
5·0000	Nil	5·0000	0·1522	3·0
7·0000	1·0683	5·9317	0·1547	2·6
10·0000	4·0320	5·9680	0·1564	2·6

TABLE VIII

Dhar yeast

Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·1884	18·8
2·0000	Nil	2·0000	0·3661	18·3
3·0000	Nil	3·0000	0·5098	17·0
4·0000	0·5952	3·4048	0·5256	15·4
5·0000	1·2500	3·7500	0·5312	14·2

TABLE IX

R. gracilis

Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·2432	24·3
2·0000	0·7521	1·2489	0·2654	21·4
3·0000	1·6240	1·3760	0·2864	20·9
4·0000	2·6300	1·3700	0·2860	20·9
5·0000	3·6486	1·3514	0·2862	21·2

EFFECT OF CONCENTRATION OF SUCROSE ON YEAST GROWTH

TABLE X

S. cerevisiæ

Amount of glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·0756	7·6
2·0000	Nil	2·0000	0·0984	4·9
3·0000	Nil	3·0000	0·1197	4·0
4·0000	Nil	4·0000	0·1386	3·5
5·0000	Nil	5·0000	0·1584	3·1
6·0000	Traces	6·0000	0·1785	3·0
7·0000	0·6536	6·3464	0·1804	2·8

TABLE XI

T. utilis

Amount of glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·0824	8·2
2·0000	Nil	2·0000	0·1022	5·1
3·0000	Nil	3·0000	0·1241	4·1
4·0000	Nil	4·0000	0·1472	3·7
5·0000	Nil	5·0000	0·1702	3·4
6·0000	0·1079	5·8921	0·1941	3·4
7·0000	0·4214	6·5786	0·2000	3·0

TABLE XII

Dhar yeast

Amount of glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·1402	14·0
2·0000	Nil	2·0000	0·3528	17·6
3·0000	Nil	3·0000	0·5922	16·4
4·0000	Nil	4·0000	0·6744	16·9
5·0000	0·9786	4·0214	0·7694	19·1

TABLE XIII

R. gracilis

Amount of glucose equivalent of sugar added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
1·0000	Nil	1·0000	0·3352	33·5
2·0000	0·2502	1·7408	0·3562	20·5
3·0000	0·5175	2·4825	0·3728	15·0
4·0000	1·5243	2·4757	0·3742	15·1
5·0000	2·5244	2·4756	0·3761	15·3

DISCUSSION

From Tables I–IV, it is clear that all the four varieties of yeasts can grow in media containing glucose, sucrose, maltose, raffinose and galactose. It was observed by Mitra and Dev Roy[9] that *Dhar yeast* could grow in

glucose and sucrose only and galactose, maltose and lactose could not be utilised by it. They also observed that *T. utilis* could not grow in media containing maltose. This difference of observations can be explained on the assumption that the capacity of the yeasts to utilize sugars is greatly influenced by the composition of media. Mitra and Dev Roy's media did not contain any vitamins of B complex group. It appears that the presence of the vitamins of B (Complex) group in our media, has activated some enzymes of the yeasts, and thus the utilization of some sugars, that could not be used under the conditions employed by Mitra and Dev Roy, has become possible.

None of the four varieties of yeasts studied by us, could utilize lactose for their growth. The component monosaccharides of lactose are galactose and glucose. The above yeasts could grow in glucose and galactose. This clearly shows that the above varieties of yeasts are not able to split lactose into glucose and galactose. Perhaps, lactose, the lactose splitting enzyme is absent from enzyme system of *S. cerevisiæ*, *T. utilis*, *Dhar yeast* and *R. gracilis*.

R. gracilis alone could grow in arabinose. The other yeasts showed no growth. This shows that yeasts are specific in utilizing different sugars for their growth.

Dhar yeast grows well in media containing ethyl alcohol, but the efficiency of growth is better at lower concentrations than at higher concentrations of alcohol.

T. utilis and *R. gracilis* did not give appreciable yields in media containing 1 per cent. V/V ethyl alcohol. At higher concentrations of alcohol in the media there was no growth at all.

S. cerevisiæ did not grow in alcohol even in lower concentrations. It is also evident from Tables VI, VII, X and XI that the efficiency of growth and yeast yield of *T. utilis* is greater than *S. cerevisiæ*. It is because of the fact that *T. utilis* is more alcohol-resistant than *S. cerevisiæ*. Moreover, it may be quite possible that *T. utilis* utilizes some alcohol, which is produced during its growth in sucrose and glucose medium, when sugar is completely utilized.

S. cerevisiæ, *T. utilis*, and *Dhar yeast*, can also grow in media containing glycerol as a source of carbon. The optimum growth and growth efficiency of different yeasts is obtained at different concentrations glucose and sucrose.

SUMMARY

The four varieties of yeasts, *S. cerevisiæ*, *T. utilis*, *Dhar yeast* and *R. gracilis* differ in their capacity to utilize, glucose, sucrose, maltose, lactose, raffinose, arabinose, ethyl alcohol and glycerol for their growth. Yeasts are highly specific in their capacity to grow in media containing different monosaccharides. The utilization of di- and trisaccharides by yeasts depend upon the capacity of a particular yeast (1) to hydrolyse the di- or trisaccharide and (2) to utilize the products of hydrolysis for their growth.

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part III. Effect of Phosphates on the Growth of *Dhar yeast* with Ethyl Alcohol as Source of Carbon

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Phosphorus is an essential constituent of all forms of plant life. The chief sources of phosphorus for yeast growth are inorganic phosphates. The classical work of Harden and Yough[1] and Wroblewski[2] have clearly established the stimulatory effect of phosphates in the zymase fermentation of glucose. According to Kalckar[3] phosphates play a very important role in the enzymatic synthesis. Besides the above, phosphorus is a constituent of yeast cells. Definite chemical compounds containing phosphorus have been isolated from fungi. Mitscherlisch[4] has reported that P_2O_5 is the predominant constituent of yeast ash.

Since most of the known varieties of yeasts are not capable of utilizing ethyl alcohol as the only source of carbon, very little is known about the role of phosphates in yeast growth and yeast metabolism, when alcohol is used as the only source of carbon. Recently, a new variety of yeast, the *Dhar yeast*, has been isolated from Indian toddy.[5] This yeast grows abundantly in media containing ethyl alcohol as the only source of carbon.[6, 7] Hence the present work was undertaken with a view to study the effect of phosphates on the growth, nitrogen balance, and phosphate intake of *Dhar yeast*, in cultures containing ethyl alcohol as the only source of carbon.

Experimental

Culture media of the following composition was prepared.

Substance	Amount in 192 c.c. of media (gm.)
Magnesium sulphate.. ..	0·0440
Calcium carbonate	0·0240
Potassium sulphate	0·1008
Ammonium sulphate ..	1·1500
Sodium chloride	0·0061
Ferrous sulphate	0·0021
Zinc sulphate	0·0250

Results

192 c.c. of the media were transferred to 500 c.c. conical flasks and in each set known amounts of potassium dihydrogen phosphate and sodium dihydrogen phosphate were added. The corresponding P_2O_5 concentration in each case was kept same. The contents of each flask were brought to pH 4·5 by dilute hydrochloric acid. The flasks were cotton-plugged and autoclaved at 15 lb. pressure for 20 minutes. After autoclaving, the flasks were cooled and to each flask 8 c.c. of absolute ethyl alcohol were added. The flasks were seeded with 0·05 c.c. of active *Dhar yeast* culture and were incubated at 35° C. for one month after which period the contents of the flasks were analysed.

Determination of the quantity of yeast was done by filtering the contents of the flasks through oven-dried weighed Whatman No. 1 filter-paper. The filter-paper together with the residue was kept in air-oven at 85° C. for 6 hours.

Estimation of total nitrogen in yeast was done according to Kjeldahl's method.[8] The total nitrogen in culture media was determined by evaporating a known volume of the filtrate with two or three drops of concentrated H_2SO_4. Further details of the procedure were same as in the Kjeldahl's method.

Estimation of P_2O_5 in yeast was done by ashing the yeast and by digesting the ash with 1 : 1 HCl. P_2O_5 in the digested mother liquor was estimated in accordance with Lorenz Reagent Method.[9]

Table I

Sodium Dihydrogen Phosphate as Source of P_2O_5

Initial P_2O_5 in culture (gm.)	Yeast grown (gm.)	P_2O_5 assimilated by yeast (gm.)	P_2O_5 in yeast (%)	Recovery of P_2O_5 by yeast (%)	Nitrogen assimilated by yeast (gm.)	Nitrogen in yeast (%)	Nitrogen left in media (gm.)
0·0000	Nil	Nil	Nil	Nil	Nil	Nil	0·2436
0·0400	0·5001	0·0112	2·24	28·00	0·0341	6·83	0·1300
0·1016	0·8164	0·0184	2·26	18·11	0·0571	7·00	0·1107
0·2024	1·2830	0·0291	2·27	14·37	0·0928	7·24	0·0814
0·3046	1·6902	0·0409	2·42	13·42	0·1237	7·32	0·0721
0·4068	1·6980	0·0475	2·81	11·67	0·1200	7·10	0·0986
0·5084	1·6900	0·0513	3·04	10·09	0·1139	6·74	0·1001
0·6100	1·6800	0·0581	3·24	9·52	0·1097	6·53	0·1241

TABLE II

Potassium Dihydrogen Phosphate as Source of P_2O_5

Initial P_2O_5 in culture (gm.)	Yeast grown (gm.)	P_2O_5 assimilated by yeast (gm.)	P_2O_5 in yeast (%)	Recovery of P_2O_5 by yeast (%)	Nitrogen assimilated by yeast (gm.)	Nitrogen in yeast (%)	Nitrogen left in media (gm.)
0·0000	Nil	Nil	Nil	Nil	Nil	Nil	0·2436
0·0400	0·5004	0·0092	1·85	23·00	0·0346	6·92	0·1326
0·1016	1·0740	0·0226	2·12	22·24	0·0652	7·01	0·1112
0·2024	1·5941	0·0343	2·18	16·81	0·1008	7·24	0·0770
0·3046	1·6150	0·0355	2·20	11·62	0·1176	7·41	0·0662
0·4068	1·6140	0·0478	2·96	11·25	0·1130	7·01	0·0706
0·5084	1·6141	0·0522	3·24	10·26	0·1100	6·82	0·0724
0·6100	1·6139	0·0552	3·42	9·04	0·1032	6·40	0·0781

DISCUSSION

It is evident that increasing doses of phosphates, bring about an increase in the growth of yeast, but this goes on upto a certain limit, beyond which there is no increase in the growth of yeast. The increase in the amount of P_2O_5 in the yeast cells is not appreciable as long as there is an increase in the growth of yeast. But when the growth of yeast becomes constant, the amount of P_2O_5 in the yeast cells rises rapidly. However at very high concentrations of phosphates the efficiency of recovery of P_2O_5 by yeast cells begins to fall.

In the beginning increasing doses of phosphates increase the percentage of nitrogen in yeast cells, but after the media attains a definite phosphate concentration, further increase in the phosphate results in decrease of nitrogen status of yeast, and more nitrogen is left in the media.

Hence, it can be concluded that phosphates are not only important in increasing the yeast yield, but to a certain extent determine phosphorus and nitrogen status of yeast.

SUMMARY

Increasing doses of phosphates have been found to increase the yeast yield, nitrogen content and phosphorus content of *Dhar yeast* in media

containing 4% ethyl alcohol V/V as the only source of carbon. The increase goes on upto a certain stage beyond which, higher doses of phosphates are not only useless but harmful in the sense that nitrogen content of the yeast begins to decrease. The efficiency of recovery of phosphates by yeast also falls down.

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STUDIES IN YEAST GROWTH UNDER NON-AERATED CONDITION

Part IV. Effect of Different Nitrogenous Compounds on Yeast Growth

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Nitrogen is an essential element for animal and plant life. It is used by fungi for functional and structural purposes. Schwann was first to point out that besides sugars yeasts required nitrogenous substances to be able to multiply and cause fermentation. In lower organisms such as yeasts and bacteria the synthesis of aromatic and heterocyclic amino acids may be accomplished and hence the protein synthesis in these organisms occur by the utilization of carbohydrates and simple sources of nitrogen.[1–3] The importance of ammonium salts in yeast growth was emphasized by Pasteur. Kayser[4] and Fernbach and Lunzenberg[5] have reported that nitrates have favourable influence in zymase fermentation. Some workers, like Kossourez have reported that yeasts can utilize nitrates, while others like Bokorny[6] do not agree with the above. He has measured the increase in the development of yeast, when nitrogen is taken from urea, and has concluded that yeasts can utilize urea as a source of nitrogen. The ability of yeasts to utilize ammonium sulphate, urea, asparagine and peptone has been used in the classification of yeasts.[7] Thorne[8] has reported that admixture of different nitrogen sources can make a special contribution to yeast growth and fermentation.

In this communication, the effect of different nitrogenous compounds as source of nitrogen for the growth of *Saccharomyces cerevisiæ*, *Torulopsis utilis*, *Dhar yeast* and *Rhodotorula gracilis*, and the effect of different concentrations of ammonium sulphate on the growth of the above yeasts have been reported.

Experimental

The methods employed for the preparation and sterilization of media, estimation of the quantity of yeast and estimation of glucose in the media are same as reported in Part II of this series. When different nitrogen compounds are used as source of nitrogen for yeast growth the amount of nitrogen present in each set is 0·0848% N.

RESULTS

Effect of Different Sources of Nitrogen

TABLE I

S. cerevisiæ

Nitrogen Source	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
KNO_2	5·0000	5·0000	Nil	Nil	Nil
$NaNO_2$	5·0000	5·0000	Nil	Nil	Nil
KNO_3	5·0000	1·7144	3·2856	0·0570	1·8
$NaNO_3$	5·0000	1·7150	3·2850	0·0586	1·8
$CO(NH_2)_2$	5·0000	Traces	5·0000	0·0806	1·6
$(NH_4)_2SO_4$	5·0000	Traces	5·0000	0·0864	1·7
NH_4Cl	5·0000	Traces	5·0000	0·0842	1·7
NH_4NO_3..	5·0000	1·1624	3·8376	0·0518	1·4
$(NH_4)_2C_2O_4$	5·0000	1·6233	3·3767	0·0448	1·3
$(NH_4)CH_3COO$	5·0000	1·6842	3·3158	0·0326	1·0

TABLE II

T. utilis

Nitrogen Source	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
KNO_2	5·0000	5·0000	Nil	Nil	Nil
$NaNO_2$	5·0000	5·0000	Nil	Nil	Nil
KNO_3	5·0000	1·0000	4·0000	0·0925	2·3
$NaNO_3$	5·0000	Nil	5·0000	0·1010	2·0
$CO(NH_2)_2$	5·0000	1·2274	3·7726	0·0826	2·2
$(NH_4)_2SO_4$	5·0000	Nil	5·0000	0·1346	2·7
NH_4Cl	5·0000	Nil	5·0000	0·1338	2·7
NH_4NO_3..	5·0000	1·3368	3·6632	0·0646	1·8
$(NH_4)_2C_2O_4$	5·0000	1·7570	3·2430	0·0568	1·8
$(NH_4)CH_3COO$	5·0000	1·7602	3·2398	0·0550	1·7

TABLE III

Dhar yeast

Nitrogen Source	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
KNO_2	5·0000	5·0000	Nil	Nil	Nil
$NaNO_2$	5·0000	5·0000	Nil	Nil	Nil
KNO_3	5·0000	4·6852	0·3148	Trace	Nil
$NaNO_3$	5·0000	4·6682	0·3318	Trace	Nil
$CO(NH_2)_2$	5·0000	3·4678	1·5322	0·1564	10·2
$(NH_4)_2SO_4$	5·0000	Nil	5·0000	0·9124	18·2
NH_4Cl	5·0000	1·1904	3·8096	0·7852	20·6
NH_4NO_3..	5·0000	0·6830	4·3170	0·5090	11·7
$(NH_4)_2C_2O_4$	5·0000	Nil	5·0000	0·6136	12·2
$(NH_4)\,CH_3COO$	5·0000	Nil	5·0000	0·6134	12·2

TABLE IV

R. gracilis

Nitrogen Source	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
KNO_2	5·0000	5·0000	Nil	Nil	Nil
$NaNO_2$	5·0000	5·0000	Nil	Nil	Nil
KNO_3	5·0000	3·7200	1·2800	0·1812	14·2
$NaNO_3$	5·0000	3·7000	1·3000	0·1842	14·2
$CO(NH_2)_2$	5·0000	4·6449	0·3551	0·0390	11·0
$(NH_4)_2SO_4$	5·0000	3·2894	1·7106	0·4707	27·5
NH_4Cl	5·0000	3·3042	1·6958	0·4700	27·5
NH_4NO_3..	5·0000	3·1665	1·8335	0·2042	11·1
$(NH_4)_2C_2O_4$	5·0000	3·4144	1·5856	0·3899	24·6
$(NH_4)CH_3COO$	5·0000	3·3789	1·6211	0·3780	23·3

Effect of Different Concentrations of $(NH_4)_2SO_4$

TABLE V

S. cerevisiæ

$(NH_4)_2SO_4$ added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
0·2500	5·0000	0·7861	4·2139	0·1171	2·8
0·3333	5·0000	0·7225	4·2775	0·1284	3·0
0·5000	5·0000	0·6410	4·3590	0·1329	3·1
1·0000	5·0000	0·9686	4·0314	0·1268	3·1
2·0000	5·0000	1·0004	3·9996	0·1260	3·2

TABLE VI

T. utilis

$(NH_4)_2SO_4$ added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
0·2500	5·0000	Nil	5·0000	0·1676	3·4
0·3333	5·0000	Nil	5·0000	0·1786	3·6
0·5000	5·0000	Nil	5·0000	0·1849	3·7
1·0000	5·0000	Nil	5·0000	0·1278	2·6
2·0000	5·0000	Nil	5·0000	0·1270	2·5

TABLE VII

Dhar yeast

$(NH_4)_2SO_4$ added (gm.)	Glucose added (gm.)	Glucose left (gm.)	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
0·2500	5·0000	0·7246	4·2754	0·5456	12·8
0·3333	5·0000	0·4672	4·5328	0·5528	12·2
0·5000	5·0000	0·1086	4·8914	0·6846	14·0
1·0000	5·0000	Nil	5·0000	0·7262	14·5
2·0000	5·0000	Nil	5·0000	0·7297	14·6

TABLE VIII

R. gracilis

$(NH_4)_2SO_4$ added (gm.)	Glucose added (gm.)	Glucose left (gm.),	Glucose used (gm.)	Yeast grown (gm.)	Efficiency (%)
0·2500	5·0000	3·2897	1·7103	0·3764	22·0
0·3333	5·0000	3·4722	1·5278	0·3577	23·5
0·5000	5·0000	3·9062	1·0938	0·3044	31·6
1·0000	5·0000	4·0322	0·9678	0·3039	31·7
2·0000	5·0000	4·0322	0·9678	0·3030	31·7

DISCUSSION

Owing to the instability of nitrites in acid solution and destructive effect of nitrous acid on proteins and aminoacids, yeasts and other fungi are not able to utilize nitrite nitrogen for their growth. Nitrites are toxic to yeasts. It is clear from the results recorded in Tables I–IV, that none of the varieties of yeasts could grow with KNO_2 or $NaNO_2$ as a source of nitrogen.

Nitrate nitrogen is also poorly used by yeasts. Pirschle[9] studied the relative value of nitrate and ammonium nitrogen for a yeast and concluded that poor utilization of nitrate nitrogen was due in part to the accumulation of nitrite in the medium. We have also observed that *Dhar yeast* when grown in potassium nitrate or sodium nitrate medium, gives a very thin scum of yeast cells at the top of the culture. The quantity of yeast produced is not appreciable. The other varieties, *viz.*, *S. cerevisiæ*, *T. utilis* and *R. gracilis*, could grow in potassium nitrate or sodium nitrate medium, but in every case the yeast yield and the efficiency of growth are much lower than what is obtained with ammonium sulphate or ammonium chloride. This can be explained on the basis of accumulation of nitrite nitrogen, that is, formed as an intermediate product, in the reduction of nitrates. This view is further supported by our observations that the growth of yeasts is lesser in ammonium nitrate medium than ammonium sulphate medium.

The growth of all the four varieties of yeasts studied by us varies in different ammonium salts such as, ammonium sulphate, ammonium chloride, ammonium oxalate and ammonium acetate. This difference is due to the different physiological effects of anions.

Wickerham[7] has reported that urea, in concentrations above 0·045%, is toxic to yeast growth. We have found that all the four yeasts could grow in medium containing 0·1818% urea.

Zakharov[10] has found that optimum amount of nitrogen for the growth of yeast strain XII is 0·0212–0·0105% nitrogen as ammonium sulphate. We have observed that with the increase of nitrogen in the medium upto 0·05% ammonium sulphate, the yeast growth goes on increasing. When the amount of ammonium sulphate in the medium is increased beyond 0·5% the growth of *S. cerevisiæ*, *T. utilis* and *R. gracilis* begins to fall. But in the case of *Dhar yeast* there is no decrease in growth, however, the increase in yeast growth is not appreciable beyond 0·5% M ammonium sulphate.

Summary

Yeasts do not grow in media containing potassium or sodium nitrite. *Dhar yeast* could not grow well in nitrate media, while *S. cerevisiæ*, *T. utilis* and *R. gracilis*, gave appreciable growth, but the efficiency of growth was lower than what is obtained with ammonium sulphate or ammonium chloride. The growth of all the varieties of yeasts is lesser in ammonium nitrate medium than in ammonium sulphate. The growth of all the varieties of yeasts varies in different ammonium salts. The optimum amount of nitrogen, required by yeasts for their growth varies with the variety of yeast. *Dhar yeast* requires maximum nitrogen.

It is concluded that yeast utilize nitrate nitrogen poorly and that the variation of yeast growth in different ammonium salts is due to the different physiological effects of the anions.

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SODIUM ALIZARIN SULPHONATE AS A COLORIMETRIC REAGENT IN INORGANIC ANALYSIS: INFLUENCE OF pH CHANGES ON THE STRUCTURE OF THE REAGENT AND COLOUR REACTIONS WITH METALLIC IONS

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Alizarin sulphonic acid known as Alizarin Red S has been found to produce colour reactions with various metallic ions. The colour produced is very sensitive with lead, copper, iron, aluminium, uranium, thorium and titanium. The change of colour with pH of Alizarin Red S has been followed spectrophotometrically and the colour has been found to change from light yellow to red and violet with progressive increase in pH. The possible structures have been discussed and the observed wave-lengths in the peaks of maximum absorption have been found to be in general agreement with those calculated by Kuhn's expression deduced from wave mechanical considerations.

HYDROXYANTHRAQUINONE dyes have been found useful as colorimetric reagents in inorganic analysis, and among them may be mentioned the uses of Alizarin, Purpurin and Quinalizarin.[1] Sodium alizarin-3-sulphonate commonly known as Alizarin Red S, has been widely used in the colorimetric determination of aluminium and various other metallic ions.[2,3,5] We have undertaken a detailed study of the coloured chelates of sodium alizarin-3-sulphonate with metals, by the spectrophotometric method, and this is a preliminary communication in the series.

Alizarin Red S is known to change its colour with the variation in the hydrogen-ion concentration and a study of the absorption spectra shows that the region of maximum absorption, λ_{max} of the dye is found to shift with the change in pH of the media. Dorta Schaeppi and coworkers[8] sought to explain the behaviour of Alizarin, from spectral studies, on the basis of the *free electron model*, developed by Kuhn.[7] Kuhn had already applied his method to explain the light absorption by several organic dyes. Later, Raghava Rao *et al.*[4,6] also applied the method to their studies with Alizarin Red S chelates.

In this paper, we have studied the influence of the variation of hydrogen-ion concentration on the colour of the dye and have attempted to explain the observations on the basis of structural changes in the dye molecule. We have also made qualitative observations on the colour formation of a number of metallic ions with Alizarin Red S. We have found that certain ions respond sensitively to the reagent, and the observation suggests the use of the reagent in the colorimetric determination of these ions, under proper conditions.

Experimental

A standard solution of Alizarin Red S (BDH Indicator) was prepared in double distilled water. To measured amounts of the solution, acid or alkali were added and total volume raised to 50 ml. The pH of the solutions were measured using Cambridge pH meter. The absorption spectra were studied using Unicam SP 500 Spectrophotometer and glass cells of 1 cm. thickness supplied with the instrument. The experiments were conducted in an air-conditioned room maintaining 28° C. The results are recorded below:

Table I

Final concentration of the dye = 0·0002 M
pH of the original solution = 4·30

Solution Number	pH of the solution
1	1·70
2	4·30
3	5·72
4	8·20
5	10·40
6	13·00

TABLE II

Absorption spectra studies of 0·0002 M sodium alizarin-3-sulphonate at various pH

Wave-length mμ	Optical density					
	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5	Solution 6
400	0·52	0·50	0·37	0·29	0·18	0·17
410	0·56	0·55	0·39	0·30	0·18	0·16
420	0·58	0·58	0·42	0·33	0·21	0·16
430	0·56	0·56	0·425	0·36	0·27	0·7
440	0·50	0·50	0·43	0·38	0·34	0·19
450	0·42	0·42	0·425	0·40	0·42	0·22
460	0·32	0·33	0·40	0·43	0·53	0·28
470	0·22	0·24	0·41	0·45	0·61	0·36
480	0·14	0·18	0·415	0·47	0·72	0·47
490	0·09	0·135	0·40	0·49	0·78	0·58
500	0·045	0·09	0·41	0·52	0·86	0·75
510	0·02	0·07	0·415	0·51	0·90	0·96
520	0·00	0·06	0·40	0·50	0·92	0·12
530	..	0·055	0·39	0·48	0·91	1·15
540	..	0·04	0·35	0·44	0·87	1·47
550	..	0·03	0·32	0·39	0·81	1·70
560	..	0·025	0·27	0·33	0·72	1·70
570	..	0·025	0·23	0·28	0·61	1·45
580	..	0·025	0·185	0·21	0·50	1·30
590	..	0·02	0·125	0·16	0·42	1·00
600	..	0·015	0·10	0·105	0·32	0·75
625	..	..	..	0·04	0·08	0·31
650	..	..	..	0·01	0·01	0·04

The results are represented graphically in Fig. 1. and summarised in Table IV.

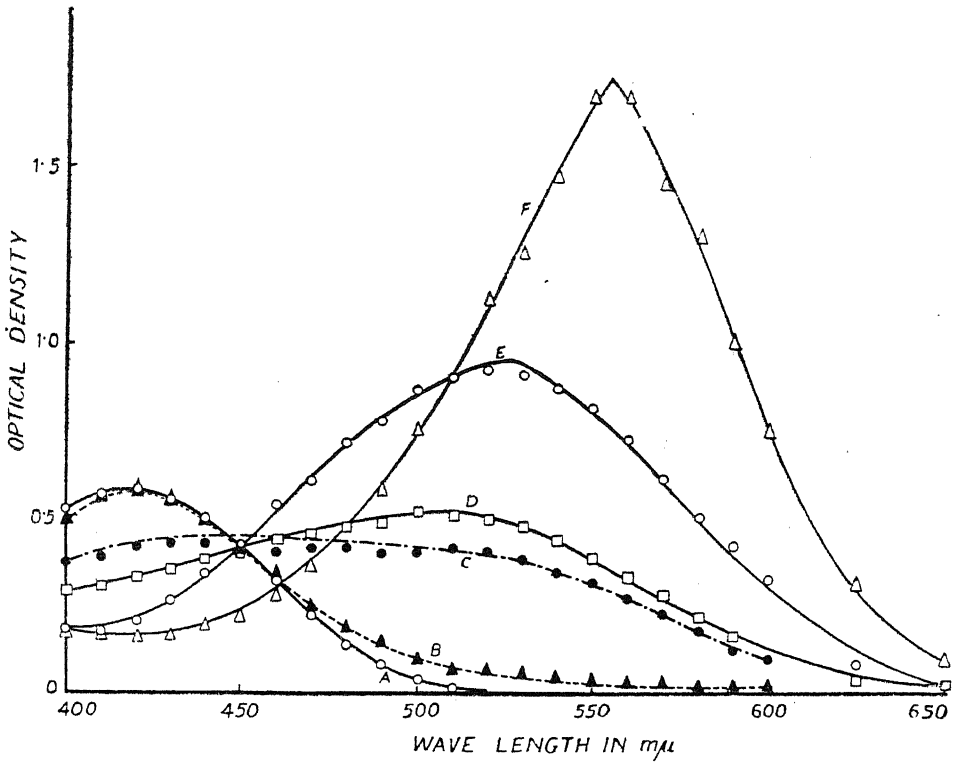


FIG. 1. Variation of optical density of Alizarin Red S with wave-length at different pH: Curve A pH 1·7; Curve B pH 4·3; Curve C pH 5·72; Curve D pH 8·2; Curve E pH 10·4; Curve F pH 13·0.

For studying colour reactions with metal ions, a 0·05% aqueous solution of Alizarin Red S was prepared. Solutions of metal salts of concentration 0·1 M were prepared in water, acid being added, where necessary, to prevent hydrolysis. In several test-tubes, 1 ml. of the solutions were taken and 2 drops of the reagent solution were added to each, and the colour was compared with an equal amount of the reagent diluted to the same extent. The table below, records the colour reactions.

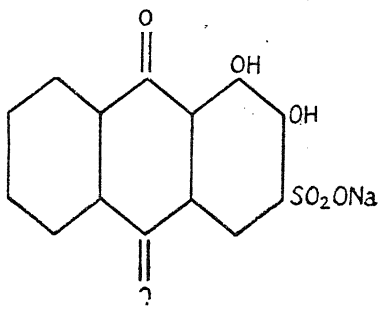
TABLE III

Salt	Colour	Remarks
1. Indicator	Yellow	..
2. $AgNO_3$	Yellow	No particular change in colour
3. $Hg_2(NO_3)_2$	Yellow	No particular change in colour
4. $Pb(NO_3)_2$	Dark reddish precipitate; purple colour on dilution	Sensitive with dilute solutions
5. $CuSO_4$	Dark violet precipitate; orange colour on dilution	Sensitive with dilute solutions
6. $CdSO_4$	Dark yellow	No particular change
7. *$Bi(NO_3)_2$	Yellow	No particular change in colour
8. *$SnCl_4$	Yellow	No particular change in colour
9. $HgCl_2$	Yellow	No particular change in colour
10. Na_3AsO_4	Red colour	..
11. *$SbCl_3$	Yellow	No particular change in colour
12. $FeCl_3$	Black precipitate; Black colour with solutions	Sensitive with dilute solutions
13. $Al(NO_3)_3$	Dark brown colour	Sensitive with dilute solutions
14. $Cr(NO_3)_3$	Yellow	No particular change in colour
15. $NiSO_4$	Brown colour	..
16. $CoSO_4$	Brown colour	..
17. $MnCl_2$	Yellow	No particular change in colour
18. $Zn(NO_3)_2$	Orange colour	..
19. $BaCl_2$	Dirty brown precipitate	..
20. $SrCl_2$	Light brown colour	..
21. $CaCl_2$	Dark violet colour	..
22. $MgSO_4$	Yellow	No particular change in colour
23. $UO_2(NO_3)_2$	Dark violet precipitate; violet colour on dilution	Sensitive with dilute solutions
24. $ThCl_4$	Red colour	Sensitive with dilute solutions
25. $TiSO_4$	Reddish brown colour	Sensitive with dilute solutions
26. Na-vanadate	Red colour	Sensitive with dilute solutions
27. Ammonium molybdate	Red colour	Sensitive with dilute solutions
28. Na-tungstate	Red colour	Sensitive with dilute solutions

* Acid added to prevent hydrolysis.

Discussion

Sodium alizarin-3-sulphonate is represented by the following structure:



The change in colour as measured by absorption spectra studies, is represented by the shift in λ_{max}, with the change in pH of the reagent, as shown in Table IV.

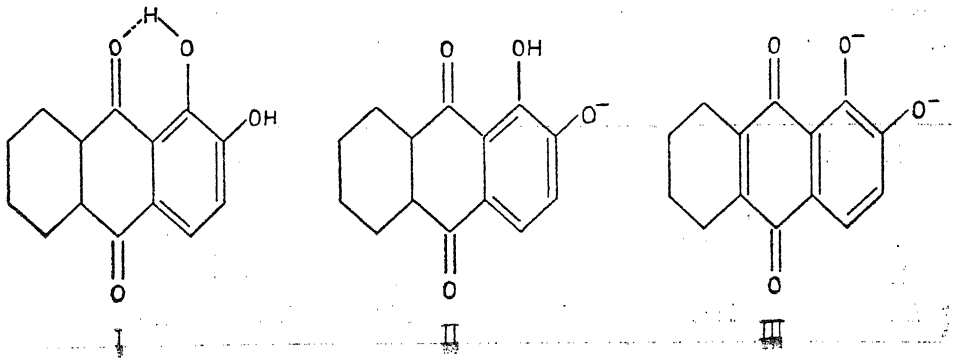
TABLE IV

Shift of λ_{max} with pH

pH	Region of maximum absorption mμ
1·7– 5·7	420
7·1–10·4	525
13·0	555

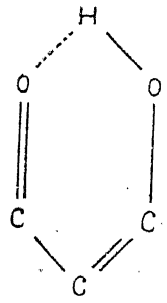
It will be evident from the above table and from Fig. 1, that Alizarin Red S exists in three ionising forms, depending upon the pH of the solution.

Raghava Rao and coworkers represented the ionisation of sodium alizarin-3-sulphonate by the following three structures:



The compound is weakly acidic in solution and with more of acid, the ionisation of hydroxylic hydrogen is suppressed and the acid form develops structure (I). Structure (II) represents the half-neutralised form and predominates between pH 7·1 to 10·4. In strongly alkaline medium both the hydrogens ionise (III).

The vibrating chains responsible for light absorption corresponding to the three ionising structures are:



I

$$HO-C=C-C=C-C=C-C=C-O^- \quad \text{(II)}$$

$$O=C-C=C-C=C-C=C-C=C-O^- \quad \text{(III)}$$

Kuhn from wave mechanical considerations arrived at the following equation for the calculation of λ_{max}:

$$\lambda = \frac{8mc}{h} \cdot \frac{L^2}{N+1}$$

where, m is the electronic mass, c the velocity of light, h Planck's constant, L the length of the vibrating chain or the free electron path and N the number of free electrons.

The values for the interatomic distances for the value of L, have been taken from Brown[10] and Palmer.[11] A close agreement has been found between the values obtained experimentally and those calculated from Kuhn's expression. The values are given in Table V.

TABLE V

Structure	L in A°	N	λ calculated mμ	λ observed mμ
I	8·16	4	436	420 at pH 1·70
II	12·15	8	537	525 at pH 10·4
III	13·58	10	548	555 at pH 13·0

Further work on metal chelates of sodium alizarin-3-sulphonate will be described in subsequent communications.

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DISSOLUTION STUDIES OF ARSENIC SULPHIDES

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In a previous communication[1] from this laboratory results have been reported on the study of thioarsenites. Various factors determining the formation of thioarsenites and their special decomposition with acids leading to the precipitation of arsenious sulphide have been investigated. During the course of investigation, it was observed that the study of the dissolution of arsenious sulphide during the formation of thioarsenites is also necessary. The present paper deals with a systematic dissolution studies of arsenic tri- and penta-sulphides, which have been followed by analytical, conductometric and potentiometric methods.

It has been found that the dissolution occurs mainly through the action of hydrosulphide ions of these sulphides forming their corresponding thioanions $(As^{III}S_4)^{5-}$, $(As^{V}S_4)^{3-}$ respectively. It proceeds very rapidly to completion as soon as sufficient HS^- ions are available to produce these thioanions, but is actually completed only when it is four times the amount of arsenic present in the system.

Experimental

A saturated solution of hydrogen sulphide was prepared at the room temperature and the sulphide estimated iodimetrically. From this solution a set of reagent mixtures were prepared in such a way that all contained the same sulphide concentration (·04 M) with varying concentrations of NaOH·50 ml. of such reagent mixtures were added to 0·0615 gm. of As_2S_3 and 0·0774 gm. of As_2S_5 respectively, *i.e.*, (containing 0·0005 gm. atom of arsenic in the reaction mixture). The mixtures were shaken well and left overnight. It was observed that with only H_2S water a yellow coloured solution appeared containing some peptized sulphide and very little dissolved substance as thioanion, but majority remained undissolved. In lower concentrations of alkali at first a yellow colour developed but after some lapse of time the solutions became colourless, leaving the separation of colloidal sulphur obtained by the oxidation of some polysulphide anions present in the mixture. In higher concentrations of alkali, the solutions remained colourless throughout, only arsenic sulphides were slowly dissolved leaving a little of unreacted sulphides and a little of sulphur.

In all cases the unreacted residues were filtered through weighed sintered glass crucibles (porosity G–4) washed thoroughly with water, alcohol and carbon disulphide successively, dried at 100–110° C. and weighed. The amounts dissolved or peptized were obtained by difference.

In another set of experiments 2 ml. of 1% $BaCl_2$ solution were added to prevent any peptization and the results thus obtained gave the amounts dissolved due to true salt formation.

All these results are graphically represented in Fig. 1, which shows that:—

1. Dissolution of these arsenic sulphides occurs mainly through the action of HS^- ions on the precipitate, and it proceeds very rapidly to completion as soon as sufficient HS^- ions are available to produce thio-anions

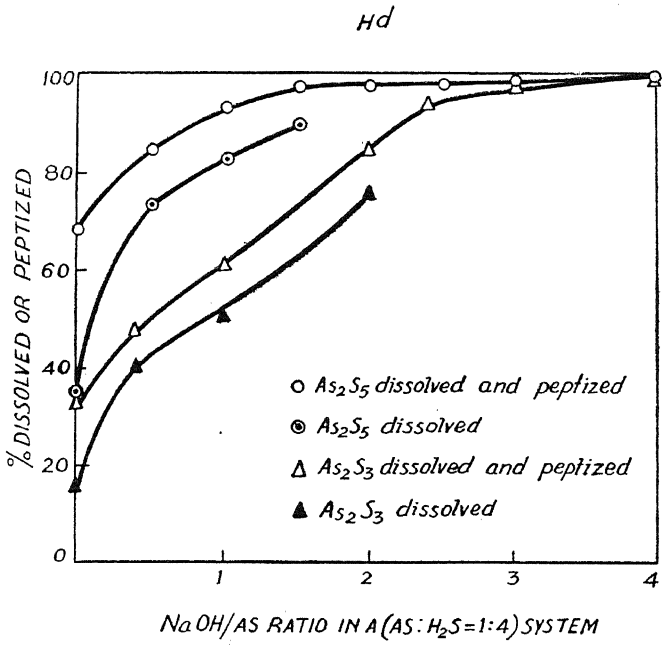

FIG. 1

$(As^{III}\ S_4)^{5-}$ and $(As^{V}\ S_4)^{3-}$ (*viz.*, where about 2·5 mols and 1·5 mols of NaHS are present per each of arsenic atom in the case of As_2S_3 and As_2S_5 respectively).

$$As_2S_3 + 5\ HS^- = 2\ AsS_4^{5-} + 5\ H^+$$

$$As_2S_5 + 3\ HS^- = 2\ AsS_4^{3-} + 3\ H^+$$

2. The H_2S molecules have got a peptizing effect on these sulphides. This dissolution due to peptization is prevented by adding barium chloride, and the second curves in each case represent the dissolutions due to true salt formation.

3. It is observed that apart from peptization effects some amount of these sulphides is dissolved in H_2S water. It is due to the HS^- ions present in H_2S water though in very minute quantity.

Conductometric Study.—The conductivities of the above mentioned reagent mixtures, NaHS solutions, and of those having arsenic sulphides dissolved in them were measured at $32° \pm \cdot 1°$ C. with a L. N. Kohlransch slide wire with an audiofrequency oscillator. The results are given in Fig. 2, which shows the following facts:—

1. The curves II and III which represent the conductivities of the reaction mixtures having As_2S_3 and As_2S_5 dissolved in them respectively, lie well below the first curve. That is the solutions become less conducting due to the formation of heavier anions.

2. Definite breaks are obtained at about 0·025 M NaOH concentration in the II and at 0·015 M conc. in the III curves. These represent a stage when so much of NaHS is formed as to give sufficient HS^- ions to produce corresponding thioanions. Thus it is in conformity with the conclusions arrived at by the analytical results.

3. The slope of the curves increases after the breaks, running more or less parallel to the first curve, showing that after the thioanions have been completely formed the further rate of increase in conductance is very nearly the same as observed for the addition of NaOH to a H_2S solution forming NaHS.

4. It should be noted that the H_2S water becomes more conducting by adding some arsenic sulphide to it.

5. It should also be observed that the III curve corresponding to As_2S_5 always lies below the II curve corresponding to As_2S_3. It can be explained in the following way:—

In the initial stages upto the NaOH conc. of 0·015 M, when a break occurs in the III curve, it lies below because (*a*) The thioarsenite anion $(As^{III}\ S_4)^{5-}$ although having the same weight as that of a thioarsenate anion $(As^{V}\ S_4)^{3-}$ has greater number of charges, hence more conducting.

(*b*) The number of heavier thioanions produced in the case of thioarsenite is less, nearly 3/5th of the thioarsenate anion at any NaOH concentration.

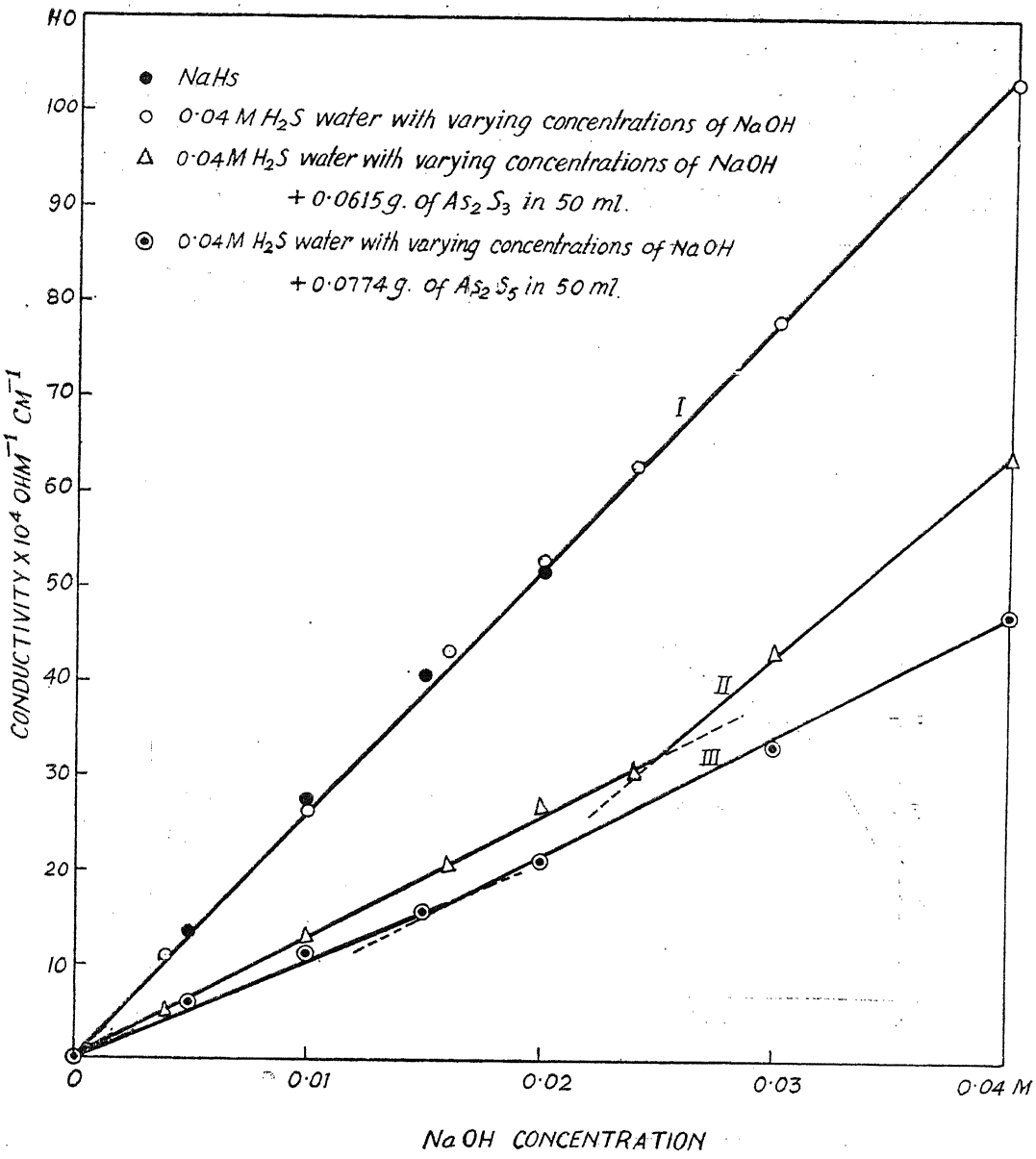

FIG. 2

After this stage the II curve remains above because of the release of greater number of hydrogen-ions in the dissolution of arsenious sulphide forming thioarsenite than in the case of arsenic pentasulphide.

pH measurements.—The pH of the above mentioned reagent mixtures and of the solutions containing arsenic sulphides dissolved in them have also

been measured by a cambridge pH meter. The results are given in Fig. 3, which shows that:—

1. The curves II and III corresponding to solutions containing respectively As_2S_3 and As_2S_5 dissolved in them, lie well below the first curve, thereby indicating conclusively that hydrogen-ions are liberated in the dissolution of these sulphides forming thioanions.

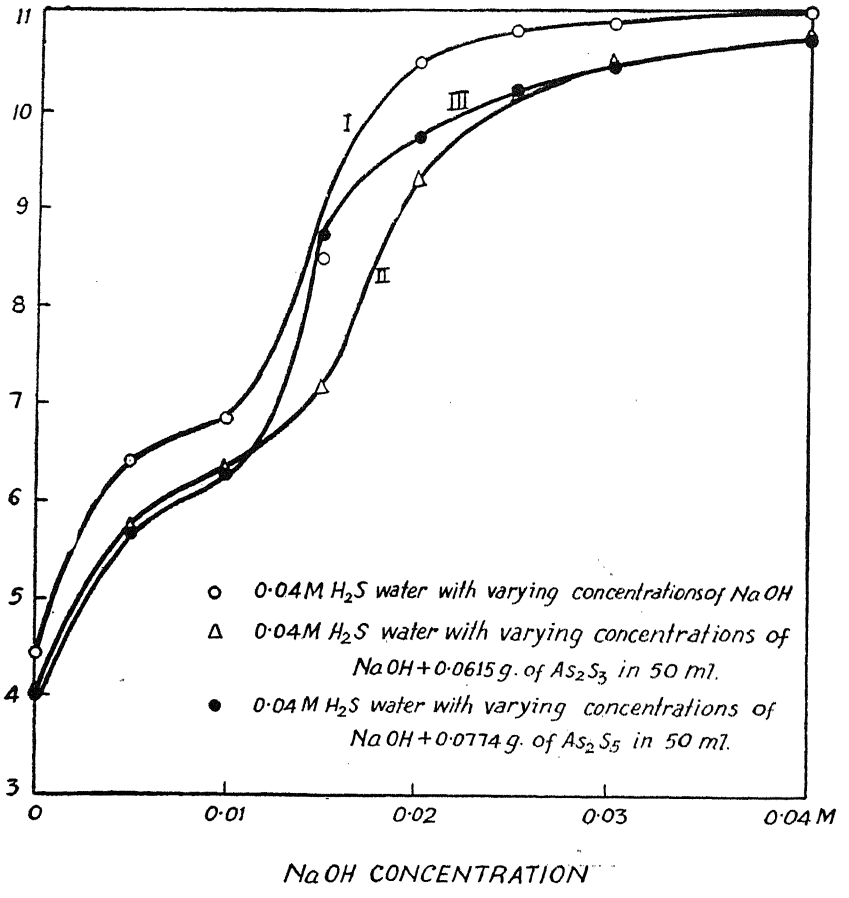

FIG. 3

2. The curves II and III in the initial stages run quite closely parallel to each other, but as the dissolution of arsenic pentasulphide approaches completion, the III curve shoots up leaving the II curve well below; finally the two curves converging and running very closely parallel to each other, as if bundled in a single line. This indicates that at first almost the same number of hydrogen-ions are produced in the both cases, but after the dissolution of As_2S_5 is complete more hydrogen-ions are being released in the

dissolution of arsenious sulphide, which is still in progress. Finally, the two curves again approach each other because of the buffering action of the excess of alkali present therein.

3. It should also be noted that both in the initial and final stages the III curve lies a little below the II curve, showing that thioarsenic acid is a bit stronger than the thioarsenious acid.

Summing up the course of the reactions at different stages can be expressed as:—

I. $As_2S_3 + 3\,HS^- = 1{\cdot}2\,(AsS_4)^{5-} + 3\,H^+ + 0{\cdot}4\,As_2S_3$

$As_2S_5 + 3\,HS^- = 2\,(AsS_4)^{3-} + 3\,H^+$

II. $As_2S_3 + 5\,HS^- = 2\,(AsS_4)^{5-} + 5\,H^+$

$As_2S_5 + 5\,HS^- = 2\,(AsS_4)^{3-} + 3\,H^+ + 2\,HS^-$

III. $As_2S_3 + 8\,HS^- = 2\,(AsS_4)^{5-} + 5\,H^+ + 3\,HS^-$

$As_2S_5 + 8\,HS^- = 2\,(AsS_4)^{3-} + 3\,H^+ + 5\,HS^-$

Summary

The dissolution of arsenic tri- and penta-sulphides in alkaline hydrogen sulphide solutions has been studied by analytical, conductometric and potentiometric methods. The results indicate that the dissolution occurs mainly through the action of HS^- ions on these sulphides forming corresponding thioanions $(As^{III}\,S_4)^{5-}$ and $(As^{V}\,S_4)^{3-}$ respectively.

$$As_2S_3 + 5\,HS^- = 2\,(AsS_4)^{5-} + 5\,H^+$$

$$As_2S_5 + 3\,HS^- = 2\,(AsS_4)^{3-} + 3\,H^+$$

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STUDIES OF HYDROUS ZINC OXIDE

Part III. Conductometric Studies on the Precipitation of the Hydrous Oxide from Zinc Sulphate Solution with Sodium Hydroxide

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Read at the 24th Annual Session of the Academy held at the University of Saugar on 28th December 1954

ABSTRACT

A number of mixtures of zinc sulphate and different amounts of alkali have been prepared to yield the ratio of Zn^{++}: OH^- from 1 : 0 to 1 : 6 and their electrical conductivities determined. The experiments have been repeated with different concentrations of the zinc solution. The conductivity has been determined (i) immediately after mixing zinc sulphate and alkali solutions and (ii) after allowing the systems to age for different periods of time.

The conductivity curves show a gradual rise up to the ratio of Zn^{++}: OH^- of about 1 : 1·5 (depending upon the concentration of zinc solution used) and then becomes constant for a short range and finally rises steeply.

With dilute solutions this ratio approaches 1 : 2. This shows that the formation of well defined basic salts seems improbable, because dilution would have favoured the formation of these and the amount of alkali needed for precipitation would be even less.

On ageing the conductivity of the systems, in general, shows an increase due to liberation of electrolytes from the precipitate by hydrolysis; with dilute solutions, however, prolonged ageing appears to favour the formation of zincates, the conductivity decreasing due to removal of alkali from the solution.

IN previous parts of the series[1, 2] we have described our studies on the precipitation of hydrous zinc oxide from zinc sulphate solution with sodium hydroxide and we have shown that association of various ions of the system goes on varying with progressive addition of alkali. It has also been observed that the adsorption of ions by the hydrous oxide during precipitation is intimately related to the hydrogen-ion concentration of the medium.

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In earlier communications[3, 4] from this laboratory, we have also carried out conductometric studies on the precipitation of several hydroxides. In this paper we have reported our results on the conductometric study of the precipitation of hydrous zinc oxide and also noted the effect of age on the conductance of the systems.

Experimental

A standard solution of zinc sulphate was prepared using A.R. B.D.H. sample and both zinc and sulphate were estimated by the usual methods and were found to be 1 : 1. A standard solution of sodium hydroxide (E. Merck sample) was prepared in water free from carbon dioxide, and standardised. In a number of 50 ml. measuring flasks, a known quantity of the zinc solution was taken and to each varying quantity of sodium hydroxide was added. The total volume was raised to 50 ml. The contents were shaken vigorously and the conductivity of the supernant liquid determined (i) immediately after mixing and after (ii) one hour, (iii) 24 hours, (iv) 168 hours, and (v) 336 hours. For the measurement of conductivity Philip's Measuring Bridge operated on 220 volts A.C. Mains with a dip type measuring cell was used. The experiments were carried out at a constant temperature of 31° C. Experiments were repeated using different concentrations of the solutions. The results are given below:—

Table I

Final Concentration of $Zn^{++} = 0 \cdot 1783$ *gm. mole/litre*

Ratio $Zn^{++} : OH^-$	Specific Conductivity $\times 10^3$ mhos				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 0	17·93	17·93	18·61	18·95	19·10
1 : 0·2052	17·50	17·42	18·36	18·38	19·10
1 : 0·4104	18·15	18·15	18·86	19·1	19·87
1 : 0·6156	17·5	18·86	19·87	19·87	20·01
1 : 0·8208	19·35	19·87	20·83	20·83	21·95
1 : 1·026	20·42	20·43	21·95	21·95	23·34
1 : 1·231	19·87	21·01	23·34	23·34	24·51
1 : 1·4364	21·01	22·28	24·51	24·51	25·8
1 : 1·6416	21·63	23·72	24·93	24·51	25·8
1 : 1·6826	22·98	24·93	25·54	25·54	26·45
1 : 1·7236	22·98	25·36	26·46	26·46	27·43
1 : 1·7646	25·38	26·27	27·34	27·75	28·28

TABLE I—(*Contd.*)

Ratio Zn^{++}: OH^-	Specific Conductivity × 10^3 mhos				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 1·8056	24·51	26·46	27·34	27·75	29·96
1 : 1·8468	24·51	27·23	27·34	28·28	30·01
1 : 2·0252	28·83	31·29	32·68	32·64	34·20
1 : 2·4624	39·13	44·56	46·56	46·54	46·54
1 : 3·078	50·73	66·82	70·04	70·04	70·96
1 : 3·6936	86·50	86·5	89·14	92·5	90·76
1 : 4·3092	104·7	108·1	119·6	111·4	108·9
1 : 4·9248	126·9	126·7	132·6	130·8	130·8
1 : 5·5404	136·2	138·9	144·2	147·1	147·1
1 : 6·156	144·3	151·7	159·8	159·8	161·2

TABLE II

Final Concentration of Zn^{++} = 0·08916 *gm. mole/Litre*

Ratio Zn^{++}: OH^-	Specific Conductivity × 10^3 mhos				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 0	10·58	10·29	10·63	10·86	10·5
1 : 0·2052	10·29	10·21	10·47	10·71	10·29
1 : 0·4104	10·50	10·58	10·98	11·14	10·66
1 : 0·6156	10·58	10·98	11·4	11·63	11·14
1 : 0·8208	11·30	11·58	12·05	12·30	11·67
1 : 1·026	13·01	12·20	12·68	12·90	12·36
1 : 1·231	14·14	13·01	13·37	13·61	13·37
1 : 1·4364	14·28	13·61	14·08	14·31	13·93
1 : 1·6416	17·30	14·42	14·56	14·97	14·36
1 : 1·6826	18·15	14·56	14·71	15·16	14·71
1 : 1·7236	15·32	14·71	15·16	15·58	15·01
1 : 1·7646	16·16	15·16	15·54	15·95	15·32
1 : 1·8056	15·81	16·16	16·34	16·27	15·58
1 : 1·8468	17·39	16·34	16·90	16·71	15·91
1 : 2·0252	18·61	18·38	18·86	18·61	17·85
1 : 2·4624	19·61	25·80	26·64	25·98	24·10
1 : 3·078	27·23	37·70	38·70	38·49	36·05
1 : 3·6936	44·56	49·68	50·70	49·681	46·54
1 : 4·3092	62·32	61·28	62·32	62·32	57·44
1 : 4·9248	70·04	70·04	70·02	73·53	68·44
1 : 5·5404	79·93	80·39	84·03	82·57	78·22
1 : 6·156	91·85	91·85	94·25	93·09	88·60

TABLE III

Final Concentration of $Zn^{++} = 0{\cdot}01783$ *gm. mole/litre*

Ratio $Zn^{++} : OH^-$	Specific Conductivity × 10^4 mhos				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 0	30·63	30·01	30·63	29·40	29·64
1 : 0·2052	30·01	30·01	30·89	29·40	29·89
1 : 0·4104	30·89	30·89	32·11	30·60	30·9
1 : 0·6156	31·84	31·97	32·97	31·56	31·97
1 : 0·8208	32·68	32·68	34·20	32·68	33·42
1 : 1·026	33·72	34·51	35·86	33·68	34·84
1 : 1·231	36·41	36·76	38·49	36·41	36·76
1 : 1·4364	37·71	38·30	39·11	37·71	38·30
1 : 1·6416	39·75	39·75	41·08	39·11	39·75
1 : 1·6826	39·97	40·85	41·77	39·11	39·75
1 : 1·7236	39·97	39·75	39·75	40·19	40·19
1 : 1·7646	40·41	40·41	40·41	40·85	40·85
1 : 1·8056	41·79	40·85	40·85	40·85	40·85
1 : 1·8468	41·30	42·02	42·02	42·26	41·77
1 : 2·0252	46·52	46·54	45·95	45·95	45·95
1 : 2·4624	58·82	57·44	56·56	56·56	54·47
1 : 3·078	79·93	78·61	76·59	75·84	73·53
1 : 3·6936	105·0	103·8	101·4	100·7	98·04
1 : 4·3092	127·2	127·2	121·4	124·6	120·5
1 : 4·9248	150·1	152·6	151·6	150·1	147·1
1 : 5·5404	175·0	178·5	177·2	177·2	171·0
1 : 6·156	196·1	203·2	200·9	201·4	198·7

DISCUSSION

In Part I of this series we have reported that complete precipitation with sodium hydroxide occurs from a zinc sulphate solution, even when the amount of alkali added is less than two equivalents. It has also been observed that as the dilution is increased, the precipitation value tends to approach the theoretical quantity. Our conductometric studies as performed in this paper show that the conductivities of the systems register a very slow rise with the progressive addition of alkali in the initial stages. When nearly 1·5 equivalents of alkali have been added to the solution of zinc sulphate of 0·1783 gm. moles per litre concentration (*vide* Table I) the conductivity begins to rise steeply with the addition of further alkali. It may be noted that with more dilute solution (*vide* Tables II and III) the steep rise commences at a later stage, which is more near the ratio of $Zn^{++} : OH^- = 1 : 2$.

These results support our previous observations reported in Part I, that with dilute solutions the amount of anion associated with the precipitate is small, and the composition of the precipitate approximates $Zn(OH)_2$, the amount of alkali required for complete precipitation being more near to 1 : 2. These results, therefore, suggest the non-validity of the hypothesis of the formation of well-defined basic salts, which would have been favoured with dilution.

The observations on the ageing of the systems are interesting. It is noted in Table I that on allowing to stand, the conductivity is, in general, raised. This shows the liberation of the absorbed electrolytes on standing. After the precipitation is complete, the hydroxide tends to adsorb alkali on account of its amphoteric nature and on ageing the particles lose their adsorptive capacity, as a consequence of which alkali is regenerated and a rise in conductivity is observed. It is interesting to note that after a period of 24 hours the precipitate becomes sufficiently aged and the systems attain equilibrium, the conductivities values remaining practically the same on further ageing.

When working with more dilute solutions the ageing effect is somewhat different, as may be seen in Tables II and III. We find that here the effect of age is less marked, than in the case of more dilute solutions. The equilibrium appears to be attained within one hour. After two equivalents of alkali have been added the conductivity values tend to show a decrease after prolonged ageing. This shows that the acid character of the hydrous oxide leads to more association of alkali with time, as a result of which the conductivity decreases.

The authors thank Professor S. Ghosh, D.SC., F.R.I.C., F.N.I., F.N.A.SC., for his kind interest in this work.

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STUDIES ON HYDROUS ZINC OXIDE

Part IV. Electrometric Studies on the Precipitation of the Hydrous Oxide

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Read at the 24th Annual Session of the Academy at the University of Saugar on 28th December 1954

ABSTRACT

The pH values of various mixtures containing Zn^{++} : OH^- from 1 : 0 to 1 : 6 have been determined. On allowing the mixtures to age, the pH values decrease for mixtures with ratio Zn^{++} : OH^- below 1 : 2, while beyond this point the pH values increase with age. This shows the liberation of acid from the precipitated hydroxide and liberation of alkali from the ion $[Zn(OH)_4]$, by hydrolysis, as shown in previous papers on the amphoterism of zinc hydroxide.

VALUABLE information can be obtained from electrometric study of precipitation of hydrous oxides. Britton[1] has carried out an extensive work on the precipitation of hydroxides by pH measurements. He has shown that a definite pH within narrow limits has to be attained before the precipitation of any particular hydroxide can commence. He arrived at this conclusion from the consideration of solubility products of insoluble hydrous oxides, according to the equation:

$$\text{Solubility product} = \frac{[K_\omega][M^{+n}]}{[H^+]^n}$$

where n is the valency of the metallic ion, $[M^{+n}]$ and $[H^+]$ are the concentrations of metallic and hydrogen-ions and K_ω is the ionic product of water. A study of the above equation reveals that for metal ions of the same valency the hydrogen-ion concentration where precipitation will occur is inversely related to the solubility product of the hydroxide. It will be of interest to note that the solubility product of ferrous, manganous, and zinc hydroxides are of the same order, but the pH at which they begin to separate are considerably different. This clearly indicates that other factors such as percentage hydrolysis of salts, specific adsorption of ions, etc., also play a significant role in the precipitation of hydrous oxides.

In previous papers of the series (2, 3, 4) we have reported our results on the precipitation and other behaviour of hydrous zinc oxide. In this

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paper, we are reporting our results on the electrometric studies on the precipitation of hydrous zinc oxide, from zinc sulphate solution with sodium hydroxide.

EXPERIMENTAL

Standard solutions of zinc sulphate and sodium hydroxide were prepared, and standardised by the usual methods. Samples of zinc sulphate and sodium hydroxide were A.R. B.D.H. and E. Merck respectively. Hydrous zinc oxide was precipitated by varying quantities of sodium hydroxide from a known quantity of zinc sulphate solution. After vigorously shaking the contents, pH of the supernatant liquid was determined immediately using an L.N. pH Indicator, operated on A.C. Mains with glass electrodes in conjunction with Calomel electrodes, supplied along with the instrument. The systems were allowed to age for (i) one hour, (ii) 24 hours, (iii) 168 hours and (iv) 336 hours, as well, and pH was determined in each case. The temperature was 31° C. Experiments were repeated using different concentrations of the solutions. The results are given below.

TABLE I

Final Concentration of $Zn^{++} = 0 \cdot 1783$ *gm. mole/litre*

Ratio $Zn^{++} : OH^-$	pH				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 0	2·60	2·60	2·60	2·50	2·42
1 : 0·2052	5·59	5·95	5·90	5·80	5·80
1 : 0·4104	6·00	6·00	6·00	5·90	5·90
1 : 0·6156	6·10	6·05	6·05	6·00	6·00
1 : 0·8208	6·30	6·20	6·10	6·08	6·08
1 : 1·026	6·25	6·20	6·20	6·16	6·18
1 : 1·231	6·45	6·35	6·35	6·30	6·30
1 : 1·4364	6·58	6·60	6·60	6·53	6·55
1 : 1·6416	7·80	7·80	7·20	7·06	7·10
1 : 1·6826	8·40	8·45	8·30	8·05	7·80
1 : 1·7236	9·00	9·15	8·35	8·05	8·05
1 : 1·7646	10·00	8·80	8·20	7·90	7·88
1 : 1·8056	9·50	9·20	8·15	7·90	7·88
1 : 1·8468	9·80	9·30	8·10	7·92	7·90
1 : 2·0252	11·40	11·55	9·80	9·40	9·32
1 : 2·4624	12·70	12·52	12·40	12·40	12·38
1 : 3·078	12·70	12·75	12·80	12·86	12·82
1 : 3·6936	13·10	12·90	12·70	13·06	13·00
1 : 4·3092	13·00	13·00	12·70	13·15	13·15
1 : 4·9248	13·10	12·95	13·30	13·30	13·28
1 : 5·5404	13·15	12·95	13·30	13·39	13·30
1 : 6·156	13·00	12·95	13·30	13·39	13·30

TABLE II

Final Concentration of Zn^{++} = 0·08916 gm. mole/litre

Ratio Zn^{++}: OH^-	pH				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 0	2·82	2·80	2·68	2·75	2·80
1 : 0·2052	6·00	6·10	5·90	5·92	6·00
1 : 0·4104	6·10	6·15	5·95	6·00	6·05
1 : 0·6156	6·20	6·20	6·00	6·08	6·10
1 : 0·8208	6·30	6·30	6·10	6·13	6·18
1 : 1·026	6·40	6·35	6·20	6·22	6·25
1 : 1·231	6·52	6·50	6·35	6·36	6·40
1 : 1·4364	6·85	6·50	6·50	6·48	6·50
1 : 1·6416	6·90	7·00	6·82	6·73	6·79
1 : 1·6826	7·25	7·00	6·90	6·88	6·89
1 : 1·7236	7·80	7·78	7·30	7·16	7·16
1 : 1·7646	7·70	7·70	7·82	7·60	7·4
1 : 1·8056	9·00	8·52	8·10	7·80	..
1 : 1·8468	9·80	8·30	8·10	7·80	7·85
1 : 2·0252	10·40	10·20	9·52	9·40	9·3
1 : 2·4624	11·85	11·53	11·85	11·80	11·6
1 : 3·078	12·50	12·58	12·55	12·50	12·49
1 : 3·6936	12·80	12·80	12·80	12·78	12·78
1 : 4·3092	12·90	12·95	12·90	12·90	12·90
1 : 4·9248	13·00	13·00	13·00	13·00	13·00
1 : 5·5404	13·10	13·10	13·10	13·05	13·10
1 : 6·156	13·10	13·12	13·10	13·10	13·13

TABLE III

Final Concentration of Zn^{++}= 0·08916 gm. mole/Litre

Ratio Zn^{++}: OH^-	pH				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 0	3·28	3·31	3·25	3·31	3·35
1 : 0·2052	6·40	6·32	6·15	6·25	6·25
1 : 0·4104	6·42	6·38	6·20	6·25	6·30
1 : 0·6156	6·43	6·38	6·28	6·35	6·42
1 : 0·8208	6·50	6·45	6·30	6·42	6·50
1 : 1·026	6·55	6·48	6·32	6·38	6·40
1 : 1·231	6·65	6·58	6·40	6·45	6·50
1 : 1·4364	6·55	6·60	6·48	6·60	6·50
1 : 1·6416	6·65	6·60	6·54	6·65	6·62

TABLE III—(*Contd.*)

Rotio $Zn^{++} : OH^-$	pH				
	Immediate	1 hour	24 hours	168 hours	336 hours
1 : 1·6826	6·90	6·61	6·51	6·70	7·70
1 : 1·7236	6·95	6·56	6·55	6·70	6·68
1 : 1·7646	7·00	6·60	6·62	7·00	6·90
1 : 1·8056	7·20	6·83	6·80	7·10	7·00
1 : 1·8468	7·10	7·20	6·92	7·10	7·10
1 : 2·0252	7·80	8·10	8·02	8·20	8·22
1 : 2·4624	9·40	9·65	9·69	9·70	9·59
1 : 3·078	10·15	10·30	10·32	10·30	10·22
1 : 3·6936	11·80	11·65	11·65	11·70	11·60
1 : 4·3092	12·20	12·10	12·08	12·00	11·93
1 : 4·9248	12·35	12·30	12·29	12·23	12·20
1 : 5·5404	12·49	12·45	12·41	12·40	12·38
1 : 6·156	12·51	12·50	12·49	12·49	12·45

DISCUSSION

The results of electrometric titrations as recorded in this paper, in general, support the conclusions arrived at from electrical conductance studies reported in Part III of the series. With concentrated solutions the inflexion in the pH curve occurs when nearly 1·5 equivalents of alkali have been added, while with dilute solutions the inflexion occurs more near to the ratio 1 : 2.

On ageing of the systems, as we find from Table I, that in the initial stages the mixtures liberate acid on ageing, while in later stages when alkali is in excess there is a tendency of the systems to become alkaline. Here also we find that the systems become sufficiently aged within 24 hours, the change in pH being insignificant after this period. In Tables II and III similar results are noted but with increase in dilution equilibrium seems to be attained even earlier. Here also the tendency of the formation of zincate is revealed in the decrease of pH of the mixtures on prolonged standing. Thus the results of the electrometric studies wholly support those obtained from electrical conductance measurements.

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STUDIES ON THE EFFECT OF AGEING OF COPPER FERROCYANIDE SOL TOWARDS ITS STABILITY CONDUCTANCE AND pH VALUE

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AGEING generally changes the degree of dispersion of the dispersed phase and also the composition of the dispersed phase and the intermicellar liquid both in the case of sol and gel. It is recognised by the changes in viscosity, surface tension, conductance, pH value and its stability towards electrolytes. The changes in the degree of dispersity of a colloidal system not only take place by variation in the size of the particle but also by the changes in the shape and structure of the dispersed particles.

In addition to the structural changes, what is more important during the process of ageing is the gradual chemical change. Tian[1] and Heyman[2] studied the ageing of colloidal solution of ferric hydroxide. According to them this process takes place in the colloidal solution in such a direction that ferric hydroxide micelles poor in chloride ions are formed by splitting of the micelles richer in chloride ions.

The experiments on the ageing of colloidal solution of copper ferrocyanide described in this paper comprise of the study of conductance; hydrogen-ion concentration and its stability towards electrolytic coagulation.

EXPERIMENTAL

Preparation of Copper Ferrocyanide Sol.—Solutions of copper sulphate and potassium ferrocyanide (A. R. Quality) were prepared in conductivity water. Assuming the colloidal particles to consist of $K_2Cu_3[Fe(CN)_6]_2$ a sol[3] of strength 1·157 grammes per litre was obtained by adding equal volumes of M/100 copper sulphate solution dropwise to M/140 potassium ferrocyanide with constant stirring. The sol was allowed to age without subjecting it to dialysis. Its conductance was found by Kohlrausch conductance bridge (Paye & Co.) and hydrogen-ion concentration was found potentiometrically and precipitation concentration of potassium chloride was determined as usual. The above three experiments were made with a freshly prepared colloidal solution. This colloidal solution was kept in a neutral

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glass Jena bottle painted with Japan black from outside and allowed to age at the room temperature. Next day the above three experiments were again repeated in an exactly similar condition. In this way the sol was allowed to age for 50 days. The above set of experiments was occasionally repeated and the results are presented in the table. The colloidal solution did not show any sign of coagulation up to a period of 50 days.

TABLE I

Age of the colloid	Conductance $\times 10^3$	pH	Precipitation conc. for KCl (for one hour)
Days			
0	1·19	6·14	30·0 milli moles/litre
1	1·20	6·08	36·6 ,, ,,
2	1·21	6·00	38·3 ,, ,,
3	1·22	5·96	39·0 ,, ,,
4	1·22	5·96	39·0 ,, ,,
25	1·27	5·80	41·2 ,, ,,
30	1·28	5·70	43·3 ,, ,,

DISCUSSION

Observation on the changes in conductance and hydrogen-ion concentration of the colloidal solution (*vide* Table) shows an increase in both, on keeping it upto a period of 50 days. From these it can be concluded that the specific surface area of the colloidal particle is decreasing; such a decrease will result in a decrease in the surface value of the particle, and thereby lessen its adsorptive capacity. On ageing the colloidal solution therefore, adsorbed ferrocyanogen ions leave the surface, and thereby increase its conductance. Again assuming that an electrical double layer is surrounding the particles, a decrease in the specific surface area will result in, due to the liberation of contra-ions from the attached part of the diffused layer which thereby decreases the pH of the solution.

Further more the results show that the changes in the conductance and hydrogen-ion concentration are comparatively much more rapid in the

beginning than in the later period. This is due to the fact that as the changes proceed further and further, the tendency of the particles to undergo these changes becomes less and less, and consequently the rates of changes as recorded before fall with the ageing. Again the constancy in the values for the three and four days aged colloidal solution as recorded here is not due to the fact that there is no change at all, but because the change has become too small to be detected. Thus after a lapse of a number of days, rather after the accumulation of the inappreciable changes into appreciable one, the changes again become detectable as indicated by observations with 25 days aged solution.

Observations on precipitation concentration for potassium chloride (*vide* Table), show an increase from 30 to 42·3 milli moles per litre on ageing the colloidal solution for 50 days. This may be explained by considering two different changes which are taking place in the colloidal particles, one being the coarsening of the particles and the other that of coagulation. Coarsening of the particles may take place throughout the period of ageing, but it is necessary to note here that coarsening alone cannot bring about its coagulation. As already seen in the experiments on the changes in conductance[4] and hydrogen-ion[5] concentration that both may increase without the actual precipitation of the colloid and it is quite likely that in this case the colloidal particles may resist the tendency of settling down, due to some internal changes in the system. These internal changes may be:— (1) Hydration, (2) Chemical change taking place in a manner as to give a layer of stabilising ions. That is the colloidal particles develop a greater capacity to form an envelope of the dispersion medium around it. This will naturally lead to a greater stability as far as its precipitation by the electrolyte is concerned. These speculations are very well borne out by the fact that the undialysed colloidal solution does not show any sign of coagulation for a period of 50 days, and above that its stability towards potassium chloride also increases.

In short it may be said that the results of the author on the ageing of colloidal solution of copper ferrocyanide go to prove that a diminution in specific surface area takes place; affecting the electrical properties of the system but together with it, the hydration of the particles may also take place, which may resist the coarsened particles to coalesce.

SUMMARY

Colloidal solution of copper ferrocyanide was prepared by adding copper sulphate solution to a potassium ferrocyanide solution with constant stirring. The colloidal solution thus prepared was allowed to age for a period of 50

days without any previous dialysis. The colloidal solution did not show any sign of coagulation during this period. Its conductance, hydrogen-ion concentration, and above all its stability towards electrolytic coagulation, increased right from the beginning up to the end of this period.

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NITROGEN TRANSFORMATIONS IN SOIL WITH MOLASSES

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Read at the 26th Annual Session of the Academy at the Aligarh Muslim University on 4th February 1957

In previous publications from this laboratories it has been shown that marked losses of nitrogen take place when ammonium salts (such as ammonium nitrate or phosphate) undergo nitrification in soil. This loss appears to be unavoidable in the soil under normal conditions of cultivation. Indeed the loss of nitrogen from artificial manures is often more than 50% as is indicated by the following observations of Russell[1]: "Crops that respond to nitrogen manuring commonly take up and fix in their tissue between one-third and one-half of the nitrogen as sulphate of ammonia, and a rather higher proportion of nitrogen added as nitrate; the remainder is lost to the crop and usually to the soil—though its fate has not been well determined."

Thus the loss is universal in the cultivated soils, and in all kinds of soils to which larger amounts of synthetic fertilizers are added, irrespective of the fact whether the soil is acidic or alkaline. Hence there ought to be some process which is common to all, and which plays the major part in the loss, other than leaching, bacterial and chemical. Dhar and co-workers[2] have conducted a large number of experiments to study the process of ammonification through oxidative deamination and nitrification and these workers have constantly emphasised that nitrification is accelerated by absorption of light, and there is more loss of nitrogen in gaseous state.

Experimental records show that attempts have been made by the soil chemists to minimise this loss of nitrogen due to denitrification process. It has been observed by several workers that addition of organic matter with inorganic fertilizer decreases the denitrification process. In addition, the presence of organic matter in the soil improves the physical texture and colloidal properties of the soil, it also enriches the soil in its humus content and trace elements. The high absorptive capacity of humus imparts the special physical properties to the soil to retain ammonium, potassium,

calcium, magnesium and other ions and liberate them slowly according to need of the plant.

The physical and chemical properties of soils vary from place to place and are dependent on the climatic conditions and the temperature of the place. Thus it is not possible for any one or even a band of workers to find out the most suitable composition of artificial fertilizer and energy-rich material which would be efficient for Indian soils in general. It is therefore very necessary that a thorough analysis and assay of the soil of a particular place must be first made, followed by experiments on nitrogen fixation and control on denitrification process in presence of inorganic fertilizers and organic matter. The results of these experiments only will enable one to recommend the use of a particular composition of inorganic fertilizer and organic matter to the farmer of that locality.

We have, therefore, in this paper, attempted to show that molasses which is often overlooked can play an important role in promoting the nitrogen status of the soil. Molasses were tried as conservator, *i.e.*, as a substance which protects the heavy loss of nitrogen from the soil when mixed with ammonium nitrate and exposed to sunlight. Observations were recorded for the loss of nitrogen, oxidation of carbon and other physical and chemical properties. Three grams of molasses per pound of the soil was taken with 50 ml. of water and was mixed with soil containing 3 grams per pound of ammonium nitrate. The sample was in duplicate in glass jars and these were exposed daily for 8 hours in sunlight.

ANALYSIS OF MOLASSES

Total carbon	31·58%
Total Nitrogen	0·5256%
Silica	6·1260%
Calcium oxide	1·38%
Phosphorus pentoxide	0·0606%
Magnesium oxide	0·0011%
Ammoniacal nitrogen	0·0010%
Nitric nitrogen	Nil

TABLE I

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Molasses

Period of Exposure	Total Carbon %	Carbon Oxidised %	Total Nitrogen %	Increase in Nitrogen %	Total NH_3—N %	Total NO_3—N %
Original Soil	0·4043	..	0·0348	..	0·0004	0·0010
0 Day ..	0·6118	..	0·0382	..	0·0004	0·0010
1 Month ..	0·5696	0·0422	0·03946	0·00126	0·0005	0·0010
2 Months ..	0·5279	0·0839	0·04098	0·00278	0·0007	0·0011
3 Months ..	0·4827	0·1291	0·04564	0·00744	0·0008	0·0011
4 Months ..	0·4387	0·1791	0·04683	0·00863	0·0010	0·0013
5 Months ..	0·3952	0·2166	0·04683	0·00863	0·0010	0·0014
6 Months ..	0·3579	0·2539	0·04641	0·00821	0·0009	0·0014
7 Months ..	0·3353	0·2765	0·04595	0·00775	0·0008	0·0015
8 Months ..	0·3133	0·2985	0·04568	0·00748	0·0008	0·0015

TABLE II

Exposed Set—Average Temperature 30° C.
One pound of Soil + 3 gm. of Molasses + 3 gm. of Ammonium Nitrate

Period of Exposure	Total Carbon %	Carbon Oxidised %	Total Nitrogen %	Decrease in Nitrogen %	Total NH_3—N %	Total NO_3—N %	% Loss
Original Soil ..	0·4043	..	0·03480	..	0·00040	0·00100	..
0 Day	0·6118	..	0·26920	..	0·15315	0·15450	..
1 Month.. ..	0·5391	0·0727	0·20537	0·06383	0·10415	0·08034	23·71
2 Months ..	0·4927	0·1191	0·20335	0·06585	0·09113	0·07262	24·46
3 Months ..	0·4589	0·1529	0·19565	0·07355	0·07811	0·06489	27·32
4 Months ..	0·4161	0·1957	0·19261	0·07659	0·07046	0·06412	28·45
5 Months ..	0·3732	0·2386	0·18873	0·08047	0·06280	0·06335	29·89
6 Months ..	0·3365	0·2753	0·18270	0·08650	0·05974	0·06181	32·13
7 Months ..	0·3121	0·2997	0·17374	0·09546	0·05667	0·06026	35·46
8 Months ..	0·2927	0·3181	0·16666	0·10254	0·05514	0·05871	38·09

TABLE III

Exposed Set—Average Temperature 30° C.

One pound of Soil + 3 gm. of Ammonium nitrate

Period of Exposure	Total Carbon %	Total Nitrogen %	Total NH_3—N %	Total NO_3—N %	% Loss
Original Soil ..	0·4043	0·0348	0·0004	0·0010	..
0 Day ..	0·4043	0·2658	0·15315	0·15450	..
1 Month ..	0·3885	0·1595	0·07636	0·08985	39·99
2 Months ..	0·3736	0·1431	0·06915	0·07881	46·16
3 Months ..	0·3591	0·1266	0·05874	0·06780	52·38
4 Months ..	0·3441	0·1152	0·05202	0·06027	56·66
5 Months ..	0·3295	0·1037	0·04527	0·05277	60·99
6 Months ..	0·3137	0·0968	0·04134	0·04797	63·58
7 Months ..	0·2980	0·0899	0·03738	0·04317	66·17
8 Months ..	0·2838	0·0792	0·03381	0·03837	70·20

DISCUSSION

A comparison of Tables II and III shows that the percentage loss of nitrogen in the case of ammonium nitrate with soil when exposed to sunlight was as heavy as 70·20% in eight months time but in the presence of molasses under similar conditions the decomposition was 38·09% in the same time. The oxidation of carbon was also greater in the case of molasses. A constant decrease in ammonia and nitrate nitrogen were observed but the decrease of ammonia was less in the case of molasses *plus* ammonium nitrate than in the case of ammonium nitrate alone with the soil.

In order to make a comparative study, molasses mixed with soil were exposed to sunlight and the carbon nitrogen changes were studied. The increase in nitrogen was not continuous. In one month the increase in nitrogen was significant, in five months time it rose to its maximum and then again a fall in nitrogen status was observed. The ammoniacal and nitrate nitrogen also increase continuously. The total increase in nitrogen was

22·5% in four months time, it was constant when analysed on fifth month and then it slowly decreased. In six months it came to 21·5%, in seventh month it was 20·3% and in eighth month it was found to be 19·4%.

The retarding effect of carbonaceous matter like sugar on the decomposition of nitrogenous compounds have been observed by many previous workers. Subrahmanyam[3] has shown that the addition of cellulosic matter is fairly effective in checking the loss of nitrogen following the addition of ammoniacal fertilizers to soils specially under Indian climatic conditions. Dhar and Mukherji[4] also obtained beneficial results of adding molasses to fields to which ammonium sulphate has been added.

It is therefore clear from the above results that the value of artificial fertilizers should be greatly enhanced if they are mixed with energy-rich materials like molasses. The greater value of organic nitrogenous compounds or a mixture of ammonium salts and carbonaceous substances for the soil than ammonium salts alone lies in the fact that not only the soil texture is improved by the colloids added with organic manure, but the carbonaceous matter added acts as an agent in the preservation of nitrogen compounds of the soil by behaving as a negative catalyst.

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CONTENTS